

## THEME [KBBE.2012.2.4-02] [Food safety and quality issues related to parasites in seafood]

Grant agreement for: Collaborative project

## Annex I - "Description of Work"

Project acronym: PARASITE

Project full title: " Parasite risk assesment with integrated tools in EU fish production value chains "

Grant agreement no: 312068

Version date: 2012-06-26

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# A1: Project summary

Project Number <sup>1</sup>	312068	Project Acronym <sup>2</sup>		PARASITE							
	One form per project										
General information											
Project title <sup>3</sup> Parasite risk assessment with integrated tools in EU fish production value chains											
Starting date <sup>4</sup>	01/02/2	01/02/2013									
Duration in months <sup>5</sup>	36	36									
Call (part) identifier 6	FP7-KB	BE-2012-6-singlesta	age								
Activity code(s) most relevant to your topic <sup>7</sup>	safety a	012.2.4-02: Food nd quality issues to parasites in									
Free keywords <sup>8</sup> Food safety, quality. parasite, seafood, risk analysis, detection methods											
		Abst	ract <sup>9</sup>								

Abstract °

Despite many efforts to ensure that only high-quality and safe products are put on the market, fish-borne parasites continue to pose risks to human health, with zoonotic infections and allergic reactions mainly following consumption of raw, lightly cooked, or marinated seafood.

The PARASITE proposal is presented by a multidisciplinary consortium of 12 European and 3 Asian research institutions and 6 European SMEs. It aims to provide new scientific evidence and technological developments to detect, monitor, and mitigate impacts of zoonotic parasites, mainly anisakid nematodes but also trematode metacercariae, occurring in European and imported fishery products. The Project will address the research needs identified by EFSA regarding the risk of seafood-borne parasites. It also will facilitate close cooperation between scientists and end-users to produce new technological solutions and management tools for both European and imported fishery products.

The Work Plan has been organized in 9 work packages, each covering different stages of a risk assessment framework, providing new epidemiological data, monitoring tools, development and implementation of parasite detection devices, technological tools for their mitigation, and dissemination of key results to all the stakeholders and the general public.

Risk assessment of zoonotic parasites will ensure significant progress beyond the state of the art. This will be achieved by improving molecular hazard identification, antigen/allergen characterization, parasite exposure assessment, detection methods and treatments for industrial and other end-users, and an integrated quantitative risk analysis based on powerful statistics and modelling,

The main results will impact by (1) contributing to enhanced seafood safety, with consequent benefits for public health and consumer confidence, (2) strengthening the competitiveness of European seafood, from the net to the plate and (3) improving EU food safety policies.

# A2: List of Beneficiaries

Project N	lumber <sup>1</sup>	312068	Project Acronym <sup>2</sup>		PARASITE		
			List of Benef	iciaries			
No	Name			Short name	Country	Project entry month <sup>10</sup>	Project exit month
1	AGENCIA ESTATAL CIENTIFICAS	CONSEJO SUPERIOR DE I	NVESTIGACIONES	CSIC	Spain	1	36
2	NASJONALT INSTIT	UTT FOR ENAERINGS-OG	SJOMATFORSKNING	NIFES	Norway	1	36
3	UNIVERSITA DEGLI	STUDI DELLA TUSCIA		UT-URS	Italy	1	36
4		E DE SECURITE SANITAIR ENT ET DU TRAVAIL	E DE L'ALIMENTATION,	ANSES	France	1	36
5	CENTRO TECNOLO	GICO DEL MAR - FUNDACI	ON CETMAR	CETMAR	Spain	1	36
6	SERVICIO MADRILE	ENO DE SALUD		SERMAS	Spain	1	36
7	HAVSTOVAN			FAMRI	Faroe Islands	1	36
8	ISTITUTO SUPERIO	RE DI SANITA		ISS	Italy	1	36
9	TRUONG DAI HOC I	NHA TRANG		IBE	Viet Nam	1	36
10	ZHEJIANG OCEAN I	JNIVERSITY		ZOUC	China (People's Republic of)	1	36
11	CENTRAL LUZON S	TATE UNIVERSITY		CLSU	Philippines	1	36
12	MAX RUBNER INST ERNAHRUNG UND	ITUT BUNDESFORSCHUNC LEBENSMITTEL	SINSTITUT FUR	MRI	Germany	1	36
13	Københavns Univers	itet		UCPH	Denmark	1	36
14	Institute of Oceanogr	aphy and Fisheries		IZOR	Croatia	1	36
15	THE UNIVERSITY C	OURT OF THE UNIVERSITY	OF ABERDEEN	UNIABDN	United Kingdom	1	36
16	LARPRO ENGINEEF	RING SL		LARPRO	Spain	1	36
17	COOPERATIVA DE A S.C.G.	ARMADORES DE PESCA D	EL PUERTO DE VIGO	ARVI	Spain	1	36
18	COMERCIAL HOSPI	TALARIA GRUPO 3 SL		CHG3	Spain	1	36
19	TECHNET GMBH G	RUNDIG + PARTNER		TNET	Germany	1	36
20	HERMES AS			HERMES	Norway	1	36

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# A2: List of Beneficiaries

No		Name	Short name	Country		Project exit month
2	21	NEDERLOF'S VISHANDEL BV	NEDERLOF'S	Netherlands	1	36

# A3: Budget Breakdown

Project Nun	Project Number <sup>1</sup> 312068     Project Acronym <sup>2</sup> PARASITE										
					One Form per Pr	oject					
Participant				Esti	mated eligible cos		Requested				
number in this project <sup>11</sup>	Participant short name	Fund. % <sup>12</sup>	Ind. costs <sup>13</sup>	RTD / Innovation (A)	Demonstration (B)	Management (C)	Other (D)	Total A+B+C+D	Total receipts	EU contribution	
1	CSIC	75.0	A	918,067.00	0.00	66,888.00	70,436.00	1,055,391.00	0.00	825,874.00	
2	NIFES	75.0	A	298,200.00	0.00	20,235.00	38,340.00	356,775.00	0.00	282,225.00	
3	UT-URS	75.0	Т	330,400.00	0.00	13,600.00	19,200.00	363,200.00	0.00	280,600.00	
4	ANSES	75.0	Т	201,600.00	0.00	8,480.00	2,496.00	212,576.00	0.00	162,176.00	
5	CETMAR	75.0	F	53,520.00	0.00	6,780.00	116,880.00	177,180.00	0.00	163,800.00	
6	SERMAS	75.0	Т	221,360.00	0.00	12,240.00	15,840.00	249,440.00	0.00	194,100.00	
7	FAMRI	75.0	Т	64,000.00	0.00	12,000.00	2,880.00	78,880.00	0.00	62,880.00	
8	ISS	75.0	Т	184,000.00	0.00	12,800.00	12,800.00	209,600.00	0.00	163,600.00	
9	IBE	75.0	Т	40,960.00	0.00	480.00	10,176.00	51,616.00	0.00	41,376.00	
10	ZOUC	75.0	F	48,720.00	0.00	360.00	7,632.00	56,712.00	0.00	44,532.00	
11	CLSU	75.0	F	48,720.00	0.00	360.00	7,632.00	56,712.00	0.00	44,532.00	
12	MRI	75.0	Т	201,920.00	0.00	13,120.00	4,224.00	219,264.00	0.00	168,784.00	
13	UCPH	75.0	Т	63,360.00	0.00	8,160.00	4,032.00	75,552.00	0.00	59,712.00	
14	IZOR	75.0	Т	89,280.00	0.00	6,160.00	1,632.00	97,072.00	0.00	74,752.00	
15	UNIABDN	75.0	Т	230,720.00	0.00	11,680.00	25,280.00	267,680.00	0.00	210,000.00	
16	LARPRO	75.0	Т	110,960.00	0.00	130,640.00	16,680.00	258,280.00	0.00	230,540.00	
17	ARVI	75.0	Т	347,200.00	0.00	10,000.00	14,000.00	371,200.00	0.00	284,400.00	
18	CHG3	75.0	Т	374,400.00	0.00	8,400.00	9,200.00	392,000.00	0.00	298,400.00	
19	TNET	75.0	Т	215,475.20	0.00	10,734.40	16,201.60	242,411.20	0.00	188,542.00	
20	HERMES	75.0	F	123,000.00	0.00	7,500.00	5,640.00	136,140.00	0.00	105,390.00	
21	NEDERLOF'S	75.0	F	115,920.00	0.00	7,500.00	5,640.00	129,060.00	0.00	100,080.00	

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# A3: Budget Breakdown

Participant				Esti	mated eligible cos	ect)		Requested		
number in this project <sup>11</sup>	Participant short name	Fund. % <sup>12</sup>	Ind. costs <sup>13</sup>	RTD / Innovation (A)	Demonstration (B)	Management (C)	Other (D)	Total A+B+C+D	Total receipts	EU contribution
Total				4,281,782.20	0.00	368,117.40	406,841.60	5,056,741.20	0.00	3,986,295.00

Note that the budget mentioned in this table is the total budget requested by the Beneficiary and associated Third Parties.

#### \* The following funding schemes are distinguished

Collaborative Project (if a distinction is made in the call please state which type of Collaborative project is referred to: (i) Small of medium-scale focused research project, (ii) Large-scale integrating project, (iii) Project targeted to special groups such as SMEs and other smaller actors), Network of Excellence, Coordination Action, Support Action.

#### 1. Project number

The project number has been assigned by the Commission as the unique identifier for your project, and it cannot be changed. The project number **should appear on each page of the grant agreement preparation documents** to prevent errors during its handling.

#### 2. Project acronym

Use the project acronym as indicated in the submitted proposal. It cannot be changed, unless agreed during the negotiations. The same acronym **should appear on each page of the grant agreement preparation documents** to prevent errors during its handling.

#### 3. Project title

Use the title (preferably no longer than 200 characters) as indicated in the submitted proposal. Minor corrections are possible if agreed during the preparation of the grant agreement.

#### 4. Starting date

Unless a specific (fixed) starting date is duly justified and agreed upon during the preparation of the Grant Agreement, the project will start on the first day of the month following the entry info force of the Grant Agreement (NB : entry into force = signature by the Commission). Please note that if a fixed starting date is used, you will be required to provide a detailed justification on a separate note.

#### 5. Duration

Insert the duration of the project in full months.

#### 6. Call (part) identifier

The Call (part) identifier is the reference number given in the call or part of the call you were addressing, as indicated in the publication of the call in the Official Journal of the European Union. You have to use the identifier given by the Commission in the letter inviting to prepare the grant agreement.

#### 7. Activity code

Select the activity code from the drop-down menu.

#### 8. Free keywords

Use the free keywords from your original proposal; changes and additions are possible.

#### 9. Abstract

10. The month at which the participant joined the consortium, month 1 marking the start date of the project, and all other start dates being relative to this start date.

11. The number allocated by the Consortium to the participant for this project.

12. Include the funding % for RTD/Innovation - either 50% or 75%

#### 13. Indirect cost model

- A: Actual Costs
- S: Actual Costs Simplified Method
- T: Transitional Flat rate
- F :Flat Rate

# Workplan Tables

Project number

312068

Project title

# PARASITE—Parasite risk assessment with integrated tools in EU fish production value chains

Call (part) identifier

#### FP7-KBBE-2012-6-singlestage

Funding scheme

Collaborative project

# WT1 List of work packages

Project N	umber <sup>1</sup>	312068	Project Ac	ronym <sup>2</sup>	PARASITE	PARASITE					
		LIST	OF WORK	PACKAGES	5 (WP)						
WP Number 53	WP Title			Type of activity <sup>54</sup>	Lead beneficiary number <sup>55</sup>	Person- months <sup>56</sup>	Start month 57	End month 58			
WP 1	ADMINIST MANAGEM	RATIVE PROJECT IENT		MGT	1	24.50	1	36			
WP 2	PARASITE	EXPOSURE ASSESS	MENT	RTD	2	148.50	1	24			
WP 3	SAMPLE A	ND DATA MANAGEM	ENT	RTD	1	47.00	1	36			
WP 4	HAZARD II	DENTIFICATION		RTD	3	75.00	3	34			
WP 5	HAZARD C	CHARACTERIZATION		RTD	6	46.25	5	36			
WP 6	IMPROVEN METHODS	MENT OF DETECTION	l	RTD	8	81.00	1	36			
WP 7	INTERVEN TO REDUC	ITIONS IN THE FOOD CE RISK	CHAIN	RTD	1	63.25	1	36			
WP 8	QUANTITA	TIVE RISK ANALYSIS	;	RTD	15	48.50	1	36			
WP 9		ON, COMMUNICATION ATION ACTIVITIES	N AND	OTHER	5	57.60	1	36			
					Total	591.60					

# WT2: List of Deliverables

Project N	umber <sup>1</sup>	31206	312068		Project	Acronym <sup>2</sup>	PARASITE			
			List of De	eliveral	oles - to	be submitted fo	r review to EC			
Delive- rable Number 61	r Deliverable Title		WP number 53	Lead benefi- ciary number		Estimated indicative person- months	Nature 62	Dissemi- nation level	Delivery date 64	
D1.1	Project Procedure Manual		1		1	3.00	R	PU	2	
D2.1	Sampling protocol for PARASITE Project		2		2	3.00	R	PU	3	
D2.2	Statistical database fo modelling	or	2		2	12.00	R	RE	18	
D3.1	Biobanking handbook		3		18	6.00	R	PU	36	
D3.2	Database o storing para		3		18	12.00	0	PU	3	
D3.3	Geo-referer web solutio		3		18	12.00	R	PP	36	
D3.4	Biobank		3		18	12.00	0	PU	36	
D4.1	Protocols fo DNA extrac		4		3	6.00	R	RE	3	
D4.2	Genetic dat report	а	4		3	12.00	R	RE	34	
D5.1	Characteriz of parasite allergens	ation	5		6	8.00	R	RE	22	
D5.2	Detrminatio of alergenic capacity of Anisakidae	;	5		6	8.00	R	RE	30	
D5.3	Parasite allergens localization		5		6	12.00	R	RE	30	
D5.4	Cellular and humural responses	b	5		6	12.00	R	RE	33	
D6.1	Prototype b on UV-micr		6		19	8.00	Р	RE	12	
D6.2	Device to te parasite in t products		6		19	8.00	Р	RE	18	

# WT2: List of Deliverables

Delive- rable Number 61	Deliverable Title	WP number 53	Lead benefi- ciary number	Estimated indicative person- months	Nature 62	Dissemi- nation level	Delivery date
D6.3	Specific primers to implement existing molecular methods	6	3	5.00	R	RE	24
D6.4	Monoclonal antibodies for detection	6	1	6.00	R	RE	36
D7.1	Report on viability and infectivity of parasites in fish and fishery products	7	1	12.00	R	PU	20
D7.2	Report on bacteria-parasite interaction in fishery products	7	2	6.00	R	PU	24
D7.3	Report on treatments for killing parasites in fishery products	7	1	8.00	R	PP	32
D7.4	Report on parasite antigen elimination or inactivation methods	7	1	8.00	R	PP	32
D7.5	Prototype for management on board of parasite contaminants	7	1	12.00	Р	PP	32
D7.6	Guideline for parasite risk management in the food chain	7	1	6.00	R	PU	36
D8.1	Report on statistical analysis of parasite abundance and genetic variability.	8	15	6.00	R	PU	28
D8.2	Report on quantitative risk assessment.	8	15	18.00	R	PU	36

# WT2: List of Deliverables

Delive- rable Number	Deliverable Title	WP number 53	Lead benefi- ciary number	Estimated indicative person- months	Nature 62	Dissemi- nation level	Delivery date 64
D8.3	Report on Willingness to Pay.	8	15	9.00	R	PU	36
D8.4	Report on Cost/Benefit scenarios Policy/Food producers.	8	16	15.00	R	PU	36
D9.1	Project website	9	5	3.00	0	PU	5
D9.2	Didactic materials for training workshops	9	5	4.00	R	RE	30
D9.3	Portfolio of technologies and analysis of market potential	9	5	6.00	R	RE	36
D9.4	Annual compilation and analysis of PARASITE's media impact.	9	5	1.00	R	PU	13
D9.5	Annual compilation and analysis of PARASITE's media impact.	9	5	1.00	R	PU	25
D9.6	Annual compilation and analysis of PARASITE's media impact.	9	5	1.00	R	PU	36
D9.7	After project action plan (roadmap)	9	5	3.00	R	PU	36
			Total	264.00			

Project Number <sup>1</sup>	312068		Project Acronym <sup>2</sup>	PÆ	ARASITE					
One form per Work Package										
Work package numbe	r <sup>53</sup>	WP1	Ту	vpe of activity <sup>54</sup>		MGT				
Work package title		ADMINISTRATIVE PROJECT MANAGEMENT								
Start month		1								
End month		36								
Lead beneficiary numb	per <sup>55</sup>	1								

Objectives

The aim of this WP is to perform an operative administrative project management by implementing the most appropriate tools and means that will guarantee a fluent exchange of information and an efficient and transparent decision-making process, including IPR management. This WP also aims to assure an efficient project reporting and a satisfactory accomplishment and follow-up of all project items, especially milestones and deliverables.

#### Description of work and role of partners

CCSIC-IIM will be in charge of the overall project coordination and management, supported by LARPRO in the administrative issues. The activities listed below are described in further detail in Section B.2.1.2.

Task 1.1. Consortium management Task Leader: CSIC IIM-E Participants : LARPRO, All partners

The coordinator will be responsible of facilitating a fluent communication among the consortium members and with the Commission, in order to guarantee the development of the work-plan as foreseen and the fulfilment of the contractual obligations to the Commission and among the consortium members.

A Consortium Agreement will be prepared and signed by all partners during the negotiation stage, prior to the entry into force of the Grant Agreement. Additionally, specific ITC-based tools such as an intranet, databases, mailing lists, on-line templates for reporting, etc., will be implemented to support the coordination and the project development follow-up.

The coordinator will also be in charge of the financial and administrative supervision and management of the project, including the distribution of the EC payments to partners.

Task 1.2. IPR management Task Leader: CSIC IIM-E Participants: LARPRO

An IPR Advisory group, who will be in charge of IPR matters, will be appointed within the Exploitation and Dissemination Committee stated in WP9.

IPR policy will be integrated in the Consortium Agreement, which will be concluded among all participants prior to the beginning of the project.

(For a detailed description of the IPR strategy, see Section B.3.2.)

The MGT work package may include scientific coordination tasks. Scientific tasks and scientific coordination tasks will be reimbursed at the "RTD/Innovation" type of activity reimbursement rate and not at the "MGT" reimbursement rate.

#### Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	CSIC	6.00
2	NIFES	0.50
3	UT-URS	0.50
4	ANSES	0.50
5	CETMAR	0.50
6	SERMAS	0.50
7	FAMRI	0.50
8	ISS	0.50
9	IBE	0.50
10	ZOUC	0.50
11	CLSU	0.50
12	MRI	0.50
13	UCPH	0.50
14	IZOR	0.50
15	UNIABDN	0.50
16	LARPRO	9.00
17	ARVI	0.50
18	CHG3	0.50
19	TNET	0.50
20	HERMES	0.50
21	NEDERLOF'S	0.50
	Total	24.50

#### List of deliverables

Delive- rable Number 61	Deliverable Title	Lead Estimated benefi- ciary person- number months		Nature 62	Dissemi- nation level <sup>63</sup>	Delivery date <sup>64</sup>
D1.1	Project Procedure Manual	1	3.00	R	PU	2
		Total	3.00			

#### Description of deliverables

D1.1) Project Procedure Manual: Project Procedure Manual, including templates and ICT tools selected to support the management process. [month 2]

#### Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead benefi- ciary number	Delivery date from Annex I <sup>60</sup>	Comments
MS1	Implementation of the internal management platform.	1	3	

Project Number <sup>1</sup>	3120	68	Project Acronym <sup>2</sup>	PA	ARASITE	
One form per Work Package						
Work package number	r <sup>53</sup>	WP2	Type of activity ⁵⁴		RTD	
Work package title		PARASITE EX	POSURE ASSESSMEN	Т		
Start month		1				
End month		24				
Lead beneficiary numb	per <sup>55</sup>	2				

#### Objectives

1. To provide comprehensive and comparable epidemiological data regarding zoonotic parasites in fish stocks originating from Major European fishing grounds.

2. To map zoonotic parasites in various fish product imports on relevant European markets

#### Description of work and role of partners

Task 2.1 Surveillance of zoonotic parasites of commercial key fish species from European fishing grounds. Task Leader: NIFES

Participants : CSIC (IIM-E), UT-URS, ANSES, FAMRI, MRI, UOC, IZOR, UNIABDN, CLSU, ARVI, HERMES, NEDERLOF'S

The epidemiological data compilation for zoonotic parasites in fish from European wild catch fisheries will include the following target fish species from four major European fishing areas (Fig. 4): herring – HE (Clupea harengus), Atlantic mackerel – MA (Scomber scombrus), blue whiting – BW (Micromesistius poutassou), European hake – EH (Merluccius merluccius), haddock – HD (Melanogrammus aeglefinus), Atlantic cod – CO (Gadus morhua), monkfish – MF (Lophius sp.), Anchovy – AN (Engraulis encrasicolus), scabbard fish – SF (Aphanopus carbo, Lepidopus caudatus) and sea bass – SB (Dicentrarchus labrax).

The primary decision criteria for the target fish species concern their importance in terms of: 1) annual consumption volume/sales value, 2) significance for the fresh fish market, 3) basis for raw or semi-raw products, 4) parasite history (e.g., RASFF notifications), and 5) key fish host species in the life-cycle of anisakid larvae in the different ecosystems/fishing grounds.

Examination procedures, sample size and parasite data recording will be harmonized among all participants according to the following template:

- Preferably freshly caught or cool stored round fish to be examined (frozen if necessary).

- Follow the main fishing season and commercial size categories (small, medium, large) for each fish species and fishing ground. Whenever feasible, sample size and general sampling procedures will follow the guidelines given in ISO 7002 and CODEX CAC/GL 50-2004, respectively. The sample size during the first sampling year will be n=180-300 per host species, sampling area and sampling round. This estimate is partly based on ANOVAs of various existing Anisakis infection data from mackerel and herring, suggesting a minimum sample size of n=60 per host size category and sampling. Due to the small body size and empirically low Anisakis infection level of Mediterranean anchovy, the sample size of this host species will be not less than 500 specimens per sampling and fishing area. However, sample size per host species and fishing area will be reevaluated after the first sampling year based on a power analysis.

Recording of location (coordinates) and date of catch, fishing gear used as well as handling-, storage- and processing procedures. Recording of fish host round wet weight, fork length, sex and/or state of maturity.
Viscera and fish sides (fillets + belly flaps) to be pressed (8-14 bar at ca 5 seconds, depending on fillet size/thickness) and subsequently frozen. Deep-frozen and subsequently thawed samples of pressed fish sides or viscera to be visually inspected under a 366 nm UV-light source.

- Host and parasite data will be recorded in spreadsheets using a common template work sheet provided by NIFES. Labelling and storage procedures for parasite or tissue samples will be harmonized, in accordance with the guidelines to be established in WP3.

Routines for coordinated reporting of data and storage information for parasite and tissue samples submitted to the PARASITE BioBank will be established, in close affiliation with WP3. The main target parasites are anisakid nematode larvae (Anisakis spp., Pseudoterranova decipiens s.l., Contracaecum spp.).

Task 2.2 Presence of zoonotic parasites in fishery product imports on European key markets: case studies Task Leader: CSIC (IIM-E)

Participants: UT-URS, ANSES, CLSU

Case study I: Preliminary research of freshly imported fillets of Nile perch to Germany.

Fillets with belly flaps of Nile perch (Lates niloticus) are imported fresh on ice to Germany. The fish originates in Lake Victoria, Africa. Data on size and weight of whole round fish will probably not be available, but data on country of origin, catching/processing date, processing plant (EU-code) and fillet size/weight will be recorded. At least 100 freshly imported fillets each year over two successive sampling years will be examined for parasites by visual inspection under a dissection microscope, and/or by artificial digestion in Pepsin/HCI. The main target parasite groups are nematodes, trematodes (metacercariae) and cestodes. Any parasites recorded will be identified to species level by classic morphology and/or molecular techniques. In the latter case, material will be sent to WP4 leader for genetic identification (Tasks 4.1.-4.2). A comprehensive literature survey on existing data will also be carried out.

Case study II: Imported Todarodes pacificus to Spain.

The short-finned squid T. pacificus is imported to Spain in lare quantitites. At least 50 specimens of T. pacificus per month during two years will be sampled. Data on dorsal mantle length and total body weight of the squid, catching/processing date will be recorded. Both sexes of these animals will be studied fresh and will be randomly selected from lots collected by the Chinese fishery. The main target parasite species will be Anisakis simplex s.l., Anisakis physeteris and Pseudoterranova decipiens. Parasites will be inspected by visual methods under a dissection microscope, and/or by artificial digestion of squid mantle in Pepsin/HCI. For genetic identification, nematode parasitic larvae will be sent to WP4 leader to proceed as follow in Task 4.1.-4.2.

Case study III: Fresh or smoked products of tuna on French and Italian markets.

Tuna is one of the most important food fish in Italy and France, and often consumed raw or smoked. Since Anisakis sp. larvae are known to occur in bluefin tuna (Thunnus thynnus), a parasitological examination of various specimens from Mediterranean fishing grounds (commercial size of 30-40 kg) will be conducted, with emphasis on the possible presence of larvae in the fish flesh/fillets. The number of fish to be examined will be  $n \ge 50$  from different fishing grounds off Italy and Croatia, and  $n \ge 50$  obtained from various French fish markets. Due to the generally high costs associated with the acquisition of tuna, the number of fish examined per sampling has to be limited in order to cover the most important places of origin/fishing grounds and catching seasons. The tuna or tuna fillets will be examined for anisakid nematode larvae by applying the UV-Press method. Additionally, considering the imports by European countries of tuna fish from the Asiatic waters, several samples ( $n \ge 50$ ) of bigeye tuna (Thunnus obesus) and the yellowfin tuna (Thunnus albacores) will be also collected from the Pacific Ocean waters of the Philippines and adjacent areas. They will be processed according to the procedures described above.

Task 2.3 Presence of zoonotic parasites in Vietnamese Pangasius production systems.

Task Leader: IBE

Participants: NIFES, UT-URS, UOC

Following two successive Sutchi catfish (Pangasianodon hypophthalmus) production cycles, 80-100 fish close to market size will be collected at least bi-monthly at different fish farms in southern Vietnam, each representing either pond-, floating cage- or net-pen enclosure facilities. To examine for the presence of zoonotic parasites with emphasis on trematode metacercariae, the flesh of the fish will be deskinned and pressed (8-10 bar) before visual inspection on a light table at low magnification. In contrast to the artificial digestion-method this procedure would allow recroding of both parasite abundance and the approximate infection site. However, the detection accuracy of the procedure will be tested by applying the artificial digestion-method on at least 20 flesh sides per sampling round and farming system. Additionally, various industrially produced fillets, ready for export, will be examined monthly over a two year period for the presence of metacercariae using the same procedures as for the freshly collected fish. The metacercariae found will be identified by applying both morphometric and molecular techniques. Finally, the infection data will be analysed and compared with respect to fish size, type of product/fillet size, farming system and time of year/season (WP8). Participant Role

NIFES WP leadership. Epidemiological data collection. Parasite investigations of cultured Pangasius species. CSIC (IIM-E) Epidemiological data collection. Case study on Pacific squid imports to Spain.

UT-URS Epidemiological data collection. Case study on fresh products of tuna in Italy.

ANSES Epidemiological data collection. Case study on fresh products of tuna in France.

FAMRI Epidemiological data collection.

IBE Parasite investigations of cultured Pangasius species.

ZOUC Parasite investigations Chinese species.

MRI Epidemiological data collection. Case study on imports of fresh Nile perch fillets to Germany.

UOC Epidemiological data collection.

IZOR Epidemiological data collection.

UNIABDN Epidemiological data collection.

CLSU Case study on tuna in Philippines

ARVI Provision of samples in western fishing areas for epidemiological data collection.

HERMES Epidemiological data collection.

NEDERLOF'S Epidemiological data collection.

#### Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	CSIC	9.50
2	NIFES	9.00
3	UT-URS	8.00
4	ANSES	8.00
7	FAMRI	4.00
9	IBE	15.00
10	ZOUC	15.00
11	CLSU	15.00
12	MRI	14.00
13	UCPH	3.00
14	IZOR	6.00
15	UNIABDN	6.00
17	ARVI	12.00
20	HERMES	12.00
21	NEDERLOF'S	12.00
	Total	148.50

#### List of deliverables

Delive- rable Number 61	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature <sup>62</sup>	Dissemi- nation level <sup>63</sup>	Delivery date <sup>64</sup>
D2.1	Sampling protocol for PARASITE Project	2	3.00	R	PU	3
D2.2	Statistical database for modelling	2	12.00	R	RE	18
		Total	15.00			

Description of deliverables

D2.1) Sampling protocol for PARASITE Project: Report on a sampling protocol for PARASITE Project [month 3] D2.2) Statistical database for modelling: Database of PARASITE Project for statistical modelling [month 18]

#### Schedule of relevant Milestones Lead Delivery Milestone benefidate from Milestone name Comments number 59 ciary Annex I 60 number Complete epidemiological data sets from 2 MS2 European wild catch fisheries, case studies 24 on fish imports

Project Number <sup>1</sup>	ject Number <sup>1</sup> 312068		Project Acronym <sup>2</sup>	PA	ARASITE	
			One	e form per Work Packa	ige	
Work package numbe	r <sup>53</sup>	WP3	Ту	pe of activity <sup>54</sup>		RTD
Work package title		SAMPLE AND	D D	ATA MANAGEMENT		
Start month		1				
End month		36				
Lead beneficiary numb	oer 55	1				

#### Objectives

1. To provide management tools for traceable and high-quality storage samples to be used in diagnosis, trials and experimental challenges within the project.

2. To implement a scientific and technological-based biobank for zoonotic parasites in fishery products.

3. To implement a computer-aid epidemiological geo-referenced database for zoonotic parasites in fish stocks and products marketed in Europe, including the development of assessment utilities for end-users.

#### Description of work and role of partners

Task 3.1. Parasite sample management.

Task Leader: CHG3

Participants: CSIC (IIM-E), NIFES, UT-URS, SERMAS

The Biobank Solution will be a non-profit service that will host a collection of parasite samples originating in the present project, organized as a technical unit defined with quality criteria, order and destination, ensuring complete traceability of the samples. The Biobank network will be composed of a central node (Central Biobank) and three sub-Biobanks offering different products and services, which will be held at IIM (CSIC), NIFES, UT-URS and SERMAS, respectively. This solution will be constructed considering some relevant EU and Member State regulations dealing with medical Biobanks and accreditation rules established by International Standards for Technological Competence (ISO 17025).

A central Biobank (central node) will be hosted in Vigo (CSIC; IIM-E) and it will manage sample and data (Fig 5). This repository will collect not only a back up of 20% of the samples of the sub-Biobanks we will create, but also the information linked to the samples (i.e., the additional information regarding to the traceability) which is contained in the sample code (2 dimensions) as an excel file. The first sub-Biobank will include whole parasites and will be established by NIFES. A sub-Biobank containing the DNA samples of all anisakid nematodes of zoonotic importance, collected from different hosts and geographical areas, will be organized by UT-URS, which also already possesses a large collection of anisakids and DNA stored that can be used in comparison with those from the present project. A third sub-Biobank will be organized at the Lab of SERMAS for the characterized antigens specific for different species of anisakid parasites of zoonotic importance, proteins and positive sera gathered from animal models after experimental infections and exposure to parasite antigens. The PARASITE Biobank solution will incorporate a adaptation of well-known available commercial software for medical biobanks (e-BANK). The computer interface will include efficient and secure control of user access, sample registration, reliable automation and connectivity, the possibility to register the location and storage of samples, easy connection between different networks, other Biobanks and sample exchange, the possibility to make advanced searches and (where relevant) compliance with data protection laws. The PARASITE Biobank will be aligned with an open public access plan post project to ensure wider use by the regulatory bodies, researchers and industry in Europe.

For storage, all the samples will be codified in tubes used 2D codification fixed at the bottom of the tube. This encryption ensures full traceability of the sample over time, while not creating extra work for the laboratory staff. This storage system is associated with reading by a scanner and software decoding to identify specific samples and export information to any ODBC database. The 2D manufacturer that guarantees 100% that no two tubes in the world will have two tubes having the same encoding.

The samples will be stored at low temperature to ensure their conservation and integrity over time (Low Temperature: refrigerators, freezers and ultra low freezers). All these systems will include Backup and Alarm

Security Systems. The average number of 2D tubes than can be stored in a single freezer for the Biobank purpose ranges from 10,000 to 50,000 for -80° and -20°C, respectively. We cannot provide the final number of samples to be stored because it is not possible to know the quantity of parasites found in the fish we will process. The central node and the three sub-Biobanks, will be permanently interconnected (see scheme). This diagram indicates the flux of samples to create the first biobank and biomolecules, and also to understand how the samples and data will be processed. A database series for management of parasitized fish stocks and products will be also implemented.

Task 3.2. Epidemiological data management

Task Leader: CSIC (IIM-E)

Participants : NIFES, UT-URS, ARVI

A comprehensive systematic literature survey on existing epidemiological data for zoonotic fish parasites will be carried out at the start of the study. This will be aided by the Report on Technological Vigilance and Intelligence provided (subcontracted by CSIC). Moreover, the epidemiological information resulting from WP2 will be incorporated in a computer interface to enable register of parasites of human health and commercial significance in those fish species of highest importance for the European market. Based on this data we will design and develop assessment utilities implemented as a web-application including demographic infection values interpretable using a "traffic light code" coding system, ArcGis risk maps, a photo gallery for parasite diagnosis based on parataxonomic criteria, and evaluation of non-conformities, recommended practices and up-to-date legislative requirements with normative indications. To that end, a data source platform within PARASITE website will be constructed to manage the datasets. This Data System will also be linked with Task 3.1.

#### Participant Role

CSIC (IIM-E) WP leadership. Management of the designed epidemiological platform with assessment utilities; participant in the Biobank structure (sub-biobank).

CHG3 Manager of Biobank solution, including node organization, infrastructure and data management NIFES Epidemiological data collection; participant in the Biobank structure (parasite sub-biobank). Implementation of assessment utilities in task 3.2.

UT-URS Epidemiological data collection; participant in the Biobank structure (DNA sub-biobank). Implementation of assessment utilities in task 3.2.

SERMAS Participant in the Biobank structure (antigen and immune sera sub-biobank) ARVI Implementation of assessment utilities in task 3.2.

#### Participant number 10 Participant short name 11 Person-months per participant 1 CSIC 14.00 2 NIFES 3.00 3 UT-URS 6.00 6 SERMAS 3.00 ARVI 3.00 17 18 CHG3 18.00 Total 47.00

#### Person-Months per Participant

#### List of deliverables

Delive- rable Number 61	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature 62	Dissemi- nation level <sup>63</sup>	Delivery date <sup>64</sup>
D3.1	Biobanking handbook	18	6.00	R	PU	36

#### List of deliverables

Delive- rable Number 61	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature 62	Dissemi- nation level <sup>63</sup>	Delivery date 64
D3.2	Database of storing parasites	18	12.00	0	PU	3
D3.3	Geo-referenced web solution	18	12.00	R	PP	36
D3.4	Biobank	18	12.00	0	PU	36
		Total	42.00			

#### Description of deliverables

D3.1) Biobanking handbook: User handbook of the Biobanking solution for the PARASITE Project [month 36]

D3.2) Database of storing parasites: Database of storing parasites, DNA samples, specific and characterized antigens and parasite specific immune sera [month 3]

D3.3) Geo-referenced web solution: Web geo-referenced solution and user handbook for management of parasitized fish stocks and products [month 36]

D3.4) Biobank: Biobank of parasite animals and biomolecules [month 36]

#### Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead benefi- ciary number	Delivery date from Annex I <sup>60</sup>	Comments
MS3	Database ready for management of parasitized fish stocks and products	18	3	
MS4	Biobank structure and infrastructure	18	6	
MS5	Internal seminar for providing skills to project Biobank users	18	7	

Project Number <sup>1</sup>	ber <sup>1</sup> 312068		Project Acronym <sup>2</sup>	PA	ARASITE	
			One	e form per Work Packa	ige	
Work package numbe	r <sup>53</sup>	WP4	Ту	pe of activity <sup>54</sup>		RTD
Work package title		HAZARD IDE	ΝΤΙ	IFICATION		
Start month		3				
End month		34				
Lead beneficiary numb	ber 55	3				

#### Objectives

1. To use genetic markers to identify and characterise species and populations of zoonotic nematode parasites infecting fish and cephalopods species and products from different geographical areas.

2. To develop new genetic markers for genotyping Anisakis species

3. To establish genes and design primers/probes to be used as "DNA barcodes".

4. To gather genetic variability data of parasites populations to be correlated to their infestation levels in order to establish scientific bases for molecular epidemiological studies of each parasite species and their populations in different geographical areas.

#### Description of work and role of partners

Task 4.1 Molecular identification, characterization and genetic structure of anisakids based on sequences analysis of the mtDNA cox2 gene.

Task leader: UT-URS

Participants: ANSES, IZOR, IBE, ZOUC, CLSU

A sub-sample (not less than 50 specimens from each selected fish and cephalopods, and geographic locality), among all the anisakids and raphidascarids recovered, will be sequenced for the mtDNA cox2 gene, in order to obtain parasites identification. These methodologies will be also applied to the parasites (anisakids) eventually recovered in fish and squid species obtained from the Asiatic region (Nile perch, Todarodes pacificus, Pangasius sp., Tilapia, Thunnus spp.). The mtDNA cox-2 gene has proved to be valuable for the identification of all the sibling species of anisakids, it is already well studied in all the anisakid nematodes by the WP4 leader and has been used by some of the other WP participants. The methodological approach to be used is very well defined and is reported in detail in Valentini et al. (2006) and Mattiucci et al.(2008, 2009). The sequences analysis of the mtDNA cox2 gene will also allow us to gather information about the genetic diversity and haplotype variability of anisakid populations and species from different geographical areas.

Task 4.2. Genetic identification to the species level of anisakid nematodes by MAE.

Task leader: UT-URS

To obtain a larger amount of anisakids identified to their species level, a large sample of all the Anisakids (Anisakis spp., Pseudoterranova spp. and Contracaecum spp.) and Raphidascarids (Hysterothylacium spp.) collected and stored at -70°C during WP2 will be delivered (frozen) to UT-URS. Genetic identification will be carried out using Multilocus Allozyme Electrophoresis (MAE) analysis. The genetic markers based on allozyme diagnostic loci, are effective, rapid and at lower cost with respect to the other genetic/molecular methodologies; they will allow the identification to the species level, of a large amount of these parasites. This is particularly needed especially when mixed infection by different species of anisakid nematodes are occurring in the examined fish and in case of very high level of infestation. A sample of anisakids collected from each sampled fish specimen, (with not less of 20-50 nematodes, in case of high infestation rate, from a single fish), will be examined. This methodology will allow, minimally, the correct identification of several thousands of the nematodes recovered in the examined fish.

The methodological approach of MAE is already well standardized by the leader of the WP4 and used as a genetic marker to detect sibling species of anisakids. The procedures applied will be those reported, in details, in Mattiucci et al., 2005, 2009.

Task 4.3. Developing new and innovative nuclear markers obtained from DNA microsatellite loci in species of the genus Anisakis.

Task leader: UT-URS

Participant: CSIC (MNCN), IZOR

The aim of this task will be the scoring of high polymorphic microsatellite nuclear genes in two species of the genus Anisakis, i.e. A. simplex sensu stricto and A. pegreffii, which are the two main species infecting fish in European waters, and which are, so far, the two anisakid species demonstrated to have a zoonotic role to humans. This approach will also be used for A. simplex C which is a species which could be identified in fish from the Asiatic basin waters.

They will be studied on several specimens (not less than 100 specimens, depending also on the polymorphic loci) of different populations of both species collected from different geographical areas and fish host species; these specimens will be previously identified to species level by using allozyme markers and/or sequenced at the mtDNA cox2 gene. The scoring of microsatellite loci in these species is already being developed by the WP4's leader. These polymorphic loci will provide new, innovative and sensitive nuclear diagnostic markers, fixed for alternative alleles, in different species of Anisakis spp., to be used not only in the genetic identification of populations and species of the two taxa. This will allow screening of the main genotypes of those species of Anisakis of zoonotic risk to humans and which infect fish hosts of commercial importance.

Task 4.4. Genetic/molecular identification of parasites of zoonotic importance (other than anisakids) recovered in fish from the Asiatic region.

Task leader: UT-URS

Participants: IBE, CLSU, ZOUC

The aim of this task will be to identify by means of molecular tools the parasites of zoonotic importance, from anisakids, recovered in the sampled fish and squids from the Asiatic region. The target genes to be used for the purpose will depend on the taxa recovered. The methodological approach will include PCR-DNA sequencing (following standard procedures) of nuclear and/or mitochondrial DNA genes and comparative analysis with sequences already available in GenBank.

Task 4.5. Sequence and population genetic analysis

Task leader: UT-URS

Participants: ANSES, IZOR, IBE, ZOUC, CLSU

The mtDNA cox2 sequences obtained in this project proposal will be compared with those previously obtained for all the anisakid species and populations so far genetically characterized by the WP4 leader, and also compared to those available and deposited in GenBank by other scientists. Estimates of genetic diversity, population genetic analysis, gene diversity, haplotype diversity, gathered from different sets of genetic data obtained from nuclear (allozymes, microsatellites) and mitochondrial markers, will be analyzed by means of appropriate programs (such as BioSYS, Genepop, CLUSTAL X, MEGA, PAUP, ARLEQUIN, NETWORK). Specific probes will be designed using the program Primer3.

Participant Role

UT-URS WP leadership. Genetic/molecular identification and characterization (by means of mtDNA cox2, allozymes, and microsatellites) (of all the anisakid and raphidascarid nematodes collected. Scoring and testing of DNA microsatellite marker loci; development of microsatellites markers; primers/probes to detect the presence of traces of the zoonotic parasites in the fish fillets; Statistical analysis of genetic data. Estimation of genetic diversity and variability data; estimation of population genetic data;

CSIC (MNCN) Testing microsatellite loci on few selected populations of A. simplex s.s. and A. pegreffii. ANSES Molecular identification, by means of mtDNA cox2 sequence analysis, of samples of anisakid and raphidascarid nematodes collected in fish from the sampling areas allocated to ANSES; DNA sequence analysis IZOR Molecular identification by mtDNA cox2 sequence analysis, of anisakid/raphidascarid nematodes, collected in fish from the sampling areas allocated to IZOR. DNA sequence analysis of DNA microsatellites in selected samples of Anisakis.

CLSU Molecular identification, by means of mtDNA cox2, of anisakid/raphidascarid nematodes collected in fish from the sampling areas allocated to Philippines. DNA sequence analysis.

ZOUC Molecular identification of anisakid nematodes by means of mtDNA cox2 collected in Todarodes pacificus from the China-Korea sampling area. DNA sequences analysis.

#### Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	CSIC	5.00
3	UT-URS	16.00
4	ANSES	7.00
9	IBE	6.00
10	ZOUC	16.00
11	CLSU	16.00
14	IZOR	9.00
	Total	75.00

#### List of deliverables

Delive- rable Number 61	Deliverable Title	Lead benefi- ciary number	nefi- indicative ry person-		Dissemi- nation level <sup>63</sup>	Delivery date <sup>64</sup>
D4.1	Protocols for DNA extraction	3	6.00	R	RE	3
D4.2	Genetic data report	3	12.00	R	RE	34
	-	Total	18.00		•	

#### Description of deliverables

D4.1) Protocols for DNA extraction: Standardized protocols for DNA extraction, PCR and sequencing of mtDNA cox2 for anisakids present in fish products. [month 3]

D4.2) Genetic data report: Report on Genetic data (sequences, genetic analysis of the data gathered from different genetic data sets, population genetics, genetic diversity and variability estimates) for anisakids present in fish products [month 34]

#### Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead benefi- ciary number	Delivery date from Annex I <sup>60</sup>	Comments
MS6	Definition of DNA primers and probes to be used in RT-PCR to detect DNA of different species of anis	3	16	

Project Number <sup>1</sup> 3120		68	Project Acronym <sup>2</sup>	PA	ARASITE	
One form per Work Package						
Work package numbe	r <sup>53</sup>	WP5	Type of activity <sup>54</sup>		RTD	
Work package title		HAZARD CHA	RACTERIZATION			
Start month		5				
End month		36				
Lead beneficiary number 55		6				

#### Objectives

1.To determine if parasites belonging to the Family Anisakidae different from Anisakis spp. that can infect the muscle of fish after migration from the coelomic cavity:

•have allergenic capacity

•are able to induce sensitization after oral administration, whether untreated or heat-treated •are specifically recognized by antibodies presented in sera from fish-eating people

2.To detect Anisakis spp. allergens in fishery products

3. To detect allergens (or potential allergens) in samples of anisakids from different regions.

4.To characterize cellular and humoural immune responses to anisakid antigens

#### Description of work and role of partners

Task 5.1 Antigen characterization for parasites other than Anisakis spp.

Task Leader: ISS

Participants : SERMAS

Crude extract and excretory/secretory (ES) antigens from Pseudoterranova spp. and Contracaecum spp. will be prepared from worms by several cycles of homogeneization-sonication followed by extraction in phosphate buffered saline. Hyper-immune sera to the parasites antigens will be obtained in rabbits using standard procedures. The antigenic profiles of the parasite extracts will be analyzed by western blotting (WB). The allergenic capacity of the different selected parasite antigens will be determined in animal models (e.g. high-immunoglobulin responder Brown Norway rats, BALB/c mice, which are able to distinguish between immunogenic proteins that trigger IgG production and allergenic proteins that induce both IgG and IgE responses). Animals will be immunized with either crude or ES antigens three times together with aluminum hydroxide as adjuvant. Sera will be evaluated by ELISA and WB. Lymphocyte proliferation and cytokine production will be evaluated according to published protocols. In parallel, laboratory animal species will be orally immunized with the selected untreated or heat-treated antigens in presence of cholera toxin or other adjuvant. The evaluation of the immune responses will be carried out as above. To determine the contact between Anisakidae parasites and human beings, by the detection of specific IgE, a panel of sera collected from fish-eating people with history of allergic reactions after fish consumption, will be screened by WB.

Task 5.2. Antigen exposure (mapping of allergens) for Anisakis spp. in fishery products.

#### Task Leader: SERMAS

#### Participants: CSIC (ICTAN)

The presence of Anisakis spp antigens able to elicit allergic reactions will be detected in fishery products with no live parasite. For this purpose, recombinant allergens will be produced and specific anti-recombinant allergen antisera will be generated in rabbits according to standard procedures. The presence of parasite antigens or allergens will be analysed in fishery products with no live larvae, that is, products which could represent an unsuspected source of parasite allergens. These products would be aquaculture fish and fishery products where parasites have only been present in parts of the fish that are not consumed (e.g. in intestines). Parasite antigens and allergens in the fishery products will be extracted by a patented method (ES2340978) with a sensitivity of 1ppm (Foodborne Pathog Dis. 2010 Aug;7(8):967-73). The method is as follow: fish fillets or fishery products will be minced 1:3 (w:v) in a Tris-buffered saline solution using an Ultra-Turrax T25 ( Janke & Kunkel IKALabortechnik, Staufen, Germany). The resulting mixture will be sonicated in a Microson ultrasonic cell

disruptor XL (Misonix, Farmingdale, NY) and centrifuged (6000 g, 20°C, 30 minutes). The pellet will be discarded and the supernatant will be acidified to pH<1 by adding 0.075mM (final concentration) HCl, incubated for 15 minutes at room temperature, neutralized to pH 7 with NaOH, and centrifuged (16000 g, 30 minutes, 20°C). The pellet was discarded and the supernatant will be analyzed for the presence of Anisakis spp antigens or allergens.

Parasite antigens and allergens will be detected and quantified by immunoblotting using anti-parasite crude extract antiserum, specific anti-recombinant allergen antisera and sera from sensitized patients. Recombinant allergens standard curves will be used for quantification.

Task 5.3 Antigen proteomics, including genetic variability.

Task Leader: CSIC (MNCN)

#### Participants: CSIC (IIM-QPM), SERMAS

Anisakis species identification from different geographical origins will be performed by applying molecular methods as described in WP4. In addition to the genetic analysis, parasite proteomic diversity will be assessed. In order to characterize the protein profiles, the species Anisakis simplex s.s. and A. pegreffii, the main species present in the fish of European waters, will be subjected to proteomic analysis. This approach will be carried out by bi-dimensional electrophoresis on a statistical sample of Anisakis spp. specimens previously identified. The procedure has already been set up for Anisakis using an Isoelectic Focusing System in our group. A reference gel will be used to identify the location of proteins so each isolated protein could be systematized. For localization of the allergens we will use the positions in the bi-dimensional gels, which will then be analyzed and compared by using proteomics software. The different described antigens of Anisakis (Ani s 1 to Ani s 12) and new allergens will be detected by western blotting with sera from Anisakis-allergic patients, as well as from patients in who the etiological agent has been identified by DNA molecular methods (such sera are already held by UT-URS and will be available for this task) in selected species or populations representing genetic variability of Anisakis spp. The representative proteins will be identified by MALDI-TOF or nano-liquid chromatography connected to ion trap mass spectrometry for peptides. Manual and computer-aided de novo sequence interpretation will be essential to identify amino acid sequences with high inter- and intra-specific variability and to characterize new allergens not previously sequenced.

Task 5.4 Characterization of the immune response to the parasite antigens

Task Leader: SERMAS

Cellular and humoral immune responses will be characterized in order to clarify the mechanisms of the adverse reactions to allergens from different species of Anisakis (mainly on A. simplex s.s., A. pegreffii and A. simplex C). Study design

Setting: patients from Comunidad Autonoma de Madrid and Comunidad de Castilla la Mancha (Spain). Subjects: 50 patients sensitised to Anisakis spp and 50 sex- and age-matched healthy controls.

Inclusion criteria: Detectable serum specific IgE anti-Anisakis spp and positive skin prick test; symtoms related to fish consumption. Healthy controls should had undetectable specific IgE anti-Anisakis spp and no history of fish allergy.

Exclusion criteria: <18 or >70 years old; pregnancy; positive specific IgE to mites, fish or prawn. Demographic data: age, sex, past and present fish consumption habits (raw, undercooked, cooked, frozen seafood).

Clinical data: type of symptoms (urticaria, angioedema, gastrointestinal symptoms, anaphylaxis, diarrhoea); number of episodes associated with fish intake; time between the onset of symptoms and the fish ingestion; allergies other than hypersensitivity to Anisakis spp; atopy; prick test to common aeroallergens, mites, fish, prawn and Anisakis spp; total IgE and specific IgE to Aniskais spp; drugs intake; clinical evolution after a diet without fish or seafood.

#### Improvement of diagnostic awareness

Skin tests and laboratory determination of specific IgE have a poor specificity for the allergy diagnosis to Anisakis spp. The analysis of the pattern recognition of parasite antigens and recombinant allergens will improve the diagnostic accuracy. Determination of specific immunoglobulins other than IgE will allow to analyse IgE non-mediated immune response to the parasite. In our experience, about 10% of patients having a suggestive history of allergy to Anisakis show very low specific IgE levels, suggesting that non-IgE mediated mechanisms are underlying their symptoms. Our team has previously reported (Parasite Immunology, 2010, 32, 67–73) that patients with predominant allergic symptoms (urticaria/angioedema or anaphylaxis) showed high levels of IL-5 and IL-4 when whole blood is incubated with an Anisakis spp crude extract. On the contrary, patients with gastrointestinal symptoms had significantly higher IFN- $\gamma$  secretion. Furthermore, some patients with severe

gastrointestinal symptoms reported a delayed skin reaction and had very low values of specific IgE (even below the cutoff values considered by some authors). These patients showed the higher levels of IFN-γ in the cellular cultures. That indicates that Anisakis spp. can induce different types of immune responses and not only IgE-mediated responses.

In order to provide tools to improve the awareness in the diagnostic of Anisakis spp allergy, we will undertake the characterization of the individual pattern of antigen and allergen recognition, the determination of different serum specific immunoglobulins and the analysis of the cytokine secretion induced by different parasite allergens. The expected results will provide a more specific serologic and/or cellular assay than the prick test and current

laboratory assays, and will help in diagnosis when IgE-mediated mechanisms are not operative. Cellular immune response: whole blood from Anisakis-allergic patients will be incubated with parasite

recombinant allergens and a parasite crude extract in order to analyze the Th1/Th2/Th17 response. IL-2,

IL-4, IL-6, IL-10, IL-17A, IFN-γ, and TNF-á will be simultaneously quantified in the culture supernatants by a cytometric bead array in a flow cytometer. SERMAS will subcontract a specialized company for the production and purification of the Recombinant allergen, which includes gene synthesis, cloning into expression vector and expression tests.

Humoral immune response: levels of specific IgE, IgG and IgG4 against parasite allergens will be determined in sera from allergic patients by an enzyme-linked immunosorbent assay. Patients' parasite allergen recognition profile will be characterized by immunoblotting. Demographic and clinical characteristics will be related with the type of immune response defined by the laboratory assays.

Cellular and humoral immune responses to Anisakis simplex will be determined at the Hospital Carlos III (SERMAS). Two Hospitals within SERMAS (Carlos III and La Paz) and another from Toledo –Spain (Hospital General Nuestra Señora del Prado) will be in charge of sample collection and clinical data register of anisakid allergic patients.

Data to be registered are those relevant to any allergy and to hypersensitivity to Anisakis simplex:

• (1) Diagnostic tests: skin prick tests, total IgE and specific IgE to Anisakis simplex, Ascaris lumbricoides and Dermatophagoides pteronyssinus.

- (2) Fish consumption: raw, frozen or well cooked fish, canned fishery products.
- (3) Allergic and/or gastrointestinal symptoms.
- (4) Severity of symptoms
- (5) Number of allergic episodes
- (6) Time interval from the last allergic episode.

Regarding implication of clinical data:

• Diagnostic tests: in order to select the study subjects. Quantification of specific IgE to Ascaris and

Dermatophagoides are used to determine possible false positive specific IgE to Anisakis by cross-reactivity (1). • Fish consumption: There are Anisakis allergic patients that do not tolerate consumption of previously frozen fish, well cooked fish or canned fish, thereafter to know the "tolerance" of the patients to eating frozen fish is a key feature to be associated with the immune response profile (2). Frequency of raw fish intake is also associated with different clinical presentation (3) and one can speculate that the type or intensity of immune response can be associated to the frequency of fish consumption.

• Allergic and/or gastrointestinal symptoms: Allergic patients can show allergic symptoms (urticaria, angioedema, anaphylaxis) and/or gastrointestinal symptoms (diarrhoea, abdominal pain). These symptoms can reflect different immune responses (4).

• Severity of symptoms, number of episodes (5) and time interval from the last episode (6) can be variables involved in the type or intensity of specific immune responses to the parasite. Changes in the type of antibody response to Anisakis during the first months from infection have been reported.

#### Participant Role

SERMAS WP leadership. Screening of human sera with antigens from species of the Anisakis simplex complex. Production of recombinant allergens and specific antisera. Antigen quantification by immunoblotting. Developing of the whole blood cultures with the parasite allergens. Developing of ELISA for quantification of specific humoral response. Inclusion of allergic patients. Sample collection. Cytokine and immunoglobulin determinations. ISS Preparation of crude extracts and secretory/excretory antigens and production of rabbit hyper immune sera from parasites other than A.simplex. Animal models. Screening of human sera with antigens from parasites other than A. simplex.

CSIC (ICTAN) Optimization of allergen/antigen extraction method. Antigen detection by immunoblotting.

CSIC (MNCN) Electrophoresis in bi-dimensional gels and western blotting. Allergen distribution in Anisakis spp. Protein variability of Anisakis spp.

CSIC (IIM-QPM) Allergen characterization and correlation with Anisakis spp and its genetic structure.

# Person-Months per Participant Participant number <sup>10</sup> Participant short name <sup>11</sup> Person-months per participant 1 CSIC 22.25 6 SERMAS 20.00 8 ISS 4.00 Total Total 46.25

#### List of deliverables

Delive- rable Number 61	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature 62	Dissemi- nation level <sup>63</sup>	Delivery date <sup>64</sup>
D5.1	Characterization of parasite allergens	6	8.00	R	RE	22
D5.2	Detrmination of alergenic capacity of Anisakidae	6	8.00	R	RE	30
D5.3	Parasite allergens localization	6	12.00	R	RE	30
D5.4	Cellular and humural responses	6	12.00	R	RE	33
		Total	40.00			

#### Description of deliverables

D5.1) Characterization of parasite allergens: Report on characterization of parasite allergens in extractive fishing products and aquaculture fish. [month 22]

D5.2) Detrmination of alergenic capacity of Anisakidae: Report on determination of the allergenic capacity of Anisakidae worms other than Anisakis species in an animal model. [month 30]

D5.3) Parasite allergens localization: Report on parasite allergens localization and relationship to genetic variability. [month 30]

D5.4) Cellular and humural responses: Report on cellular and humoral responses to the Anisakid parasites [month 33]

#### Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead benefi- ciary number	Delivery date from Annex I <sup>60</sup>	Comments
MS7	Characterization of the risk exposure to allergens in fishery products with no alive parasites	6	26	
MS8	Characterization of the mechanisms of allergic sensitisation and exposure to Anisakids	6	36	

#### Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead benefi- ciary number	Delivery date from Annex I <sup>60</sup>	Comments
MS9	Proteomics identification of differentially expressed relevant antigens	6	36	

Project Number <sup>1</sup> 3120		312068		Project Acronym <sup>2</sup>	PÆ	ARASITE
One form per Work Package						
Work package number	r <sup>53</sup>	WP6	Ту	/pe of activity <sup>54</sup>		RTD
Work package title		IMPROVEMENT OF DETECTION METHODS				
Start month		1				
End month		36				
Lead beneficiary number 55		8				

#### Objectives

To improve visual, ultraviolet and molecular inspection methods for detection of parasites of human health significance in fishery products, making them usable by industry, research bodies and sanitary authorities.

#### Description of work and role of partners

Task 6.1. Improvement of the visual inspection scheme for detection of parasites of human health significance in fish fillets. Development of a new technical device to test the viability of parasites in processed fish products Task Leader: MRI

Participants: NIFES, CSIC (IIM-E), ANSES, TNET

Commission Regulation (EC) N° 853/2004 (see also EC N° 2074/2005), states that... "Food business operators must ensure that fishery products have been subjected to a visual examination for the purpose of detecting visible parasites before being placed on the market. They must not place fishery products that are obviously contaminated with parasites on the market for human consumption". The aim of this task is the evaluation of the accuracy of the visual inspection currently mandated by the EC. To this end, the statistical relationship between the number of detectable larvae in the abdominal cavity and the number of larvae in the edible part of fish (i.e., the muscles) will be determined. Moreover, the efficacy of the washing practices to remove nematode larvae from the peritoneal linings of the abdominal cavity and from edible organs (gonads, liver), will be investigated. The penetration rates (ratio of the number of larvae detected in the muscle to the total number of larvae detected) will be recorded. One hundred specimens of each targeted fish species from 10 fishing areas (as defined in WP 2) will be analyzed.

Moreover, in order to prove the safety of fish products produced under typical processing conditions applied in the European fish industry, a low cost viability test device for Anisakid nematodes will be developed. A computer-image system will be used to record the life status of individual nematodes, isolated from the final products. Anisakids will be activated in pepsin/HCI and visualized by monochromatic light of suitable wave length. A time series of 2D-images of contour patterns will be recorded by camera and combined to make a 3D-activity image which will provide information about the life status of the nematodes. The test system is aimed to be used by control laboratories and in food processing industry. The PARASITE Biobank will provide the parasite samples from these trials with a certified scheme for species identification and storage.

Task 6.2. Technological enhancement of the UV-Press method for mass screening of parasites in fishery products.

Task Leader: CSIC (IIM-E),

Participants: MRI, NIFES, CHG3

An on-site technical device based on the UV-Press method has been already developed (Levsen and Lunestad, 2010). To improve this system, spectral analysis of live and dead nematodes from different Anisakidae species will be carried out using a Leica Microsystem Confocal Microscopy TCS-SP2-AOBS with various UV-lasers. This will allow the generation of appropriate scanner settings to characterize the emission spectrum profiles of nematode larvae in fresh, frozen, heated, marinated, salted, and micro-waved fish. Additionally, the spectrum characterizations of nematode larvae will be recorded in fish which have been minimally processed by the fish processing industry. These technologies including high hydrostatic pressure (HHP), modified atmosphere packaging (MAP), or electrolyzed oxidizing (EO) water; prove to be effective in de- or re-vitalizing parasites. These treatments will be carried out in fish samples, mainly fillets, spiked with Anisakidae larvae as described before. Furthermore, the efficacy of the treatments will be evaluated using appropriate commercial systems

for the generation of HHP (Quintus Food Press 35L-600), MAP (Technotrip MAP machine) and EO (Envirolyte EL-400 for EO).

The biological basis for fluorescence in different anisakid species will be studied using lambda-scan sections and the stack profile statistics measured in regions of interest across the entire image series. This will allow us to select the optimal excitation wavelength exhibited by nematodes and fish muscle and to get the best peak separation of fluorescence intensity under each specific fish condition and treatment. The emission bandwidth will be further used in device design to achieve the optimal contrast in images acquired during fish nematode inspections.

A new automated LED lighting system for anisakid larva detection will be developed by refining the existing UV-device which has been proven to give outstanding colour images with excellent contrast. To analyse the UV-images, a validated computer-image system with large sampling capacity in real-life ship and plant operations will be developed by CHG3. To that end, simulation models will be provided to determine the optimal lambda scans under different fish handling scenarios. In addition to the required specificity and sensitivity, criteria of low cost, simplicity, rapidity, stability will be also evaluated. The PARASITE Biobank will provide all parasite samples with a certified scheme for species identification, and storage.

Task 6.3. Implementation of molecular methodology based on Real Time-PCR to detect parasites and/or their traces in fishery products.

Task Leader: UT-URS

Participants: NIFES, ANSES

The RT-PCR assay targeting the mitochondrial cytochrome oxidase II (mtDNA cox-2) will allow, at a low concentration of DNA, detection and identification, to species level, of all anisakid nematodes which occur in fish fillets. This gene has been recently used for the molecular/genetic characterization of all species of Anisakis, Pseudoterranova and Contracaecum of zoonotic importance (Mattiucci et al., 2008, 2009) and for Hysterothylacium, which has not been considered to have zoonotic importance, but often co-infects the same fish species. The methodology will include the design of a RT-PCR primers/probe for anisakid parasites, PCR optimization and the determination of the detection limit. The PARASITE Biobank will participate in this task with a certified scheme for species identification and storage.

Task 6.4. Development of immune assays to detect parasites and/or their traces in fishery products.

Task Leader: CSIC (MNCN)

Participants: CSIC (IIM-QPM), ISS

To detect anisakid parasites and/or their traces, representative proteins (result of WP5), considered to be markers for species of anisakids will be identify by MALTI-TOF or nano-liquid chromatography connected to ion trap mass spectrometry. This study will be extended to other anisakids provided by the Biobank (WP3). Monoclonal antibodies to the identified proteins or peptides will be raised. Synthetic peptides of at least 12 to 20 amino acids will be used as antigens, bearing a cysteine in the extreme of each peptide to be carried through maleimide (MBS, Pierce), to the haemocyanic protein (KLH, Pierce), used due to its highly immunogenic property. The monoclonal antibodies obtained will be characterized and their use will be evaluated in different immunoassays aimed to detect and quantify anisakid proteins in biological samples. Their potential use for commercial purposes will be evaluated.

Task 6.5. Validation of the developed and/or implemented methods and evaluation of their performance by Ring Trials.

Task Leader: ISS

Participants: NIFES, ANSES, CSIC (IIM-E), MRI, UT-URS

The RT-PCR and UV methods will be subjected to an inter-laboratory trial to demonstrate their suitability for the industrial use. For this purpose reference materials from the PARASITE Biobank as well as from the European Union Reference Laboratory for Parasites and the UT-URS will be used. The digestion method will be considered the confirmatory method for the UV-microtech development. Specificity, sensitivity and repeatability of each method will be determined. The efficiency in parasite detection of the methods will be evaluated. The most sensitive, specific and repeatable method(s) will be selected to organize a Ring Trial involving at least five experienced laboratories to evaluate reproducibility of the test/s and reliability of data produced by each laboratory.

Task 6.6. Beta-testing of validated detection methods at industrial level. Task Leader: CETMAR Participants: ARVI, HERMES, NEDERLOF'S

A beta-testing workshop will be held to transfer the methods to the participating laboratories and SMEs. Qualified personnel will illustrate the improved methods, using proof-of-concepts and providing means for testing and introducing beneficial changes in their own safety management systems. Reference parasite samples will be provided by the PARASITE Biobank. ARVI will subcontract a lab company specialized in DNA assays to participate in the beta-testing of molecular diagnostic methods.

#### Participant Role

ISS WP leadership. Ring trial organization and immune assays NIFES Improving UV-methodology and Ring trial participation MRI Viability test, Improving UV methodology and Ring trial participation UT-URS Improving molecular methodology and Ring trial participation, CSIC (IIM) Improving UV methodology and Ring trial participation CSIC (MNCN) Development of immune detection assays CETMAR Beta-testing organization and tutorial series to SMEs ANSES Improving molecular technologies and Ring trial participation TNET Device development to test parasite viability CHG3 Biobank facilities and technological enhancement of the UV Press method ARVI Beta-testing participation (UV microtech and molecular methods) HERMES Beta-testing participation (UV microtech) NEDERLOF'S Beta-testing participation (UV microtech)

#### Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	CSIC	14.00
2	NIFES	4.00
3	UT-URS	5.00
4	ANSES	4.00
5	CETMAR	5.00
8	ISS	4.00
12	MRI	4.00
17	ARVI	7.00
18	CHG3	6.00
19	TNET	16.00
20	HERMES	6.00
21	NEDERLOF'S	6.00
	Total	81.00

#### List of deliverables

Delive- rable Number 61	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature 62	Dissemi- nation level <sup>63</sup>	Delivery date <sup>64</sup>
D6.1	Prototype based on UV-microtech	19	8.00	Р	RE	12
D6.2	Device to test parasite in fish products	19	8.00	Ρ	RE	18

#### List of deliverables

Delive- rable Number 61	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature 62	Dissemi- nation level <sup>63</sup>	Delivery date 64
D6.3	Specific primers to implement existing molecular methods	3	5.00	R	RE	24
D6.4	Monoclonal antibodies for detection	1	6.00	R	RE	36
Υ		Total	27.00		~	~

#### Description of deliverables

D6.1) Prototype based on UV-microtech: Prototype based on UV-microtech to count anisakids in fish products [month 12]

D6.2) Device to test parasite in fish products: Technical device to test the viability of parasites in processed fish products [month 18]

D6.3) Specific primers to implement existing molecular methods: Specific primers to implement existing molecular methods to detect anisakids and raphidascarids (and their traces) in fish products [month 24]

D6.4) Monoclonal antibodies for detection: Monoclonal antibodies for the detection of anisakids in fish products [month 36]

#### Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead benefi- ciary number	Delivery date from Annex I <sup>60</sup>	Comments	
MS10	Improved methods ready for harmonization	8	26		
Project Number <sup>1</sup> 312068		Project Acronym <sup>2</sup>	PA	ARASITE	
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		One form per Work Packa	age		
Work package numbe	r <sup>53</sup>	WP7	Type of activity 54		RTD
Work package title		INTERVENTIONS IN THE FOOD CHAIN TO REDUCE RISK			
Start month		1			
End month		36			
Lead beneficiary numb	oer 55	1			

#### Objectives

1.To assess the viability and infectivity of anisakids in commercial products under different treatments and conditions.

2.To obtain evidences on the interactions between parasites and bacteria in the flesh of post-harvest fish under different storage conditions.

3.To design optimal treatments for the inactivation of anisakids in fishery products.

4. To develop specific treatments to reduce or inactivate the allergenic capacity of anisakids.

5.Development of an on-board prototype to kill zoonotic anisakids in fish offals.

### Description of work and role of partners

Task 7.1 Variation of the viability and infectivity of parasites in fishery products Task Leader: CSIC (IIM-E) Participants: CSIC (ICTAN), CSIC (MNCN), UT-URS, ANSES, UOC, ARVI

7.1.1. Determination of the viability and infectivity as a function of species of anisakids in commercial fish: In vitro and in vivo approaches will be tested to assess Anisakid viability and infectivity in the most relevant fish species and hosts from epidemiological and statistical points of view (see WP2). For in vitro studies, parasite samples will include A. pegreffii collected from Mediterranean horse mackerel (Trachurus mediterraneus) and anchovy (Engraulis encrasicolus); A. simplex s.s. from Lemon sole (Solea senegalensis) and cod (Gadus morhua); A. pegreffii and A. simplex s.s. from hake (Merluccius merluccius) and blue whiting (Micromesistius poutassou)]; Pseudoterranova decipiens and Contracaecum osculatum from Baltic cod (Gadus morhua). Samples will be obtained from ARVI and MercaMadrid. Genetic identification of samples will be obtained as defined in WP4. Since most of the standard techniques used to assess parasites viability are not satisfactory for all treatments or conditions (Rodriguez Mahillo et al, 2008; Vidacek et al, 2009 a, b, c), for in vitro tests we will select the most efficient methods among: (a) Dye exclusion (trypan blue exclusion test), (b) Fluorogenic dyes (fluorescein diacetate, propidium iodide), (c) Fluorescence probes (Mito Tracker, Lyso Tracker, SBTI), (d) the UV test (based on autofluorescence of Anisakids at excitation wavelength of 366 nm), and (e) enzymatic activities (esterases, arilamidases, phosphatases). The new technical device developed in WP6 to test the viability of parasites in processed seafood will be used when available. Nematode samples will also be analysed by Scanning Electron Microscopy (SEM), Environmental SEM (ESEM) and optical microscopy in order to describe their morphological alterations. Composition of the Anisakid cuticles will be studied in samples prepared as for ESEM, using an an integrated system analysis with an energy dispersive spectrometer and wavelength dispersive X ray spectrometer coupled with the FEIQuanta 200 microscopy (Oxford Instruments, Oxford, UK) (Tejada et al., 2006). Identification of elements will be made with reference to a database of internal standards provided by Oxford Instruments with INCA platform software.

To study their infectivity, parasite larvae including Contracaecum osculatum, Anisakis simplex s.s. and Pseudoterranova decipiens (third stage larvae) will be isolated from tissues of Baltic cod Gadus morhua caught in the Southern Baltic Sea (ICES subdivisions 24-26) applying methods as described in Skov et al. (2009) Subsamples of recovered worms will be forwarded to UT-URS for molecular identification. In order to evaluate infectivity and pathogenicity to mammals of these worms, experimental infection studies will be designed. These will use mice and hamsters kept at the University of Copenhagen, Section of Biomedicine, under standard conditions (room temperature, 12:12 dark-light cycle, fed ad libitum). In addition, the infectivity to fish will be examined by performing experimental infections of rainbow trout. Groups of five animals will be used in each

study. Following anaesthetizing the rodent (or fish) a total of three freshly recovered worms (third stage larva) will be placed into the stomach of the host. Euthanasia will be performed at 1, 2, 4 and 6 days post-infection. Parasites will be located, pathological changes recorded and tissue affected will be preserved in neutral formalin for histopathology and immunohistochemistry (IHC) of host tissue. The developmental stage of the worm will be determined. IHC performed will follow methodology described in Olsen et al. (2011).

7.1.2. Assessment of infectivity of Anisakids after treatments given under suboptimal conditions: The infectivity of Anisakids found in commercial fish will be investigated with live parasite larvae exposed to selected treatments in conditions (i.e. time-temperature) that affect the intactness and motility of the larvae but are not sufficient to kill them. This will be compared with untreated larvae. The treatments will be carried out either in isolated larvae or in artificially infected fish and the methodology used will be as in task 7.1.1.

7.1.3. Passage through transport hosts: The research concerning the effects of anisakids and related excretory secretory products (ES) on passage through transport hosts aims to demonstrate the variability in parasite and ES depending on the microenvironment provided by the host. An experimental oral challenge will be conducted. Healthy, hatchery-bred fishes will be transferred into experimental tanks for two weeks prior to the experiment feeding on commercial pellets to satiation. Additionally, L3 larvae of A. simplex s.s. and A. pegreffii will be isolated from the body cavities of fishes known as hosting a single parasite species. According to literature, in Norwegian spring spawning herring A. simplex s.s. is the only sibling species present. As to A. pegreffi, they will be collected from Mediterranean anchovies. In any case, after the experiment, the larvae used for that experiment will be then identified genetically by the appropriate molecular markers. Then, larvae will be cleaned in PBS, and commercial feed pellets used as the carrier for parasite larvae in the challenge experiments with sea breams, turbots and rainbow trout (25 each plus 25 feeding on pellets without any worms as a control). The fishes will be monitored for 8 h after feeding to ensure they did not regurgitate any pellets and then reared in the experimental tank for 35 days feeding on uninfected commercial feed pellets. At that time, fish will be sacrificed and dissected. Then, he number of L3 Anisakis, their condition (dead or alive), the site of infection and the surrounding host tissue for revealing parasite ES products by immunohistochemistry will be recorded.

Task 7.2 Interactions between parasites and bacteria in the flesh of post harvest fish under different storage conditions.

Task Leader: NIFES

Participants: CSIC (IIM-E), CSIC (MNCN)

Parasites bacteria interactions during post mortem storage. The otherwise sterile flesh of freshly harvested fish may become contaminated by bacteria found in or on muscle-invading parasites such as the larvae of some species of Anisakid nematodes. The intestinal contents of fish contain bacteria in high numbers, which may be transported into the fish flesh by Anisakid larvae transiently passing the fish host's intestine. The larvae's migration may thus result in an additional microbial load in the fish flesh, possibly affecting the quality and remaining shelf life of the products. The main component of the task is to describe the microbial population in muscle invading parasites and in the fish flesh by combining conventional cultivation-based methods and molecular methods (PCR/DDGE/sequencing). Moreover, the spatial distribution of microbes in or on actual anisakid species will be described with the aid of advanced microscopy. The spoilage potential of parasite-borne bacteria in various fishery products will be examined in suitable experimental models. Having high abundance of parasites in the muscle, blue whiting from Northeast-Atlantic fishing grounds will be chosen as the primary model organism.

### Task 7.3 Treatments for inactivation of Anisakids in fishery products

Task Leader: CSIC (ICTAN)

### Participants: CSIC (IIM-E), CSIC (MNCN), ARVI

7.3.1. Evaluate the optimal thermal treatments (freezing and heating) in relation to Anisakid species and fish host: The EU and US regulations on thermal treatments given to fishery products differ in terms of time and temperature conditions. As for frozen fish products, no information is available on the effects of freezing rate in the most frequently used commercial freezers (i.e. air blast, plate, or liquid nitrogen freezers) or by newer freezing methods such as high pressure shift freezing (PSF) or cell alive system (CAS). The study of the effect of freezing rate and novel freezing systems is therefore crucial in order to test their effects on the viability of larvae in conditions of maximum quality of the fish products. These techniques will be examined in the present task. Although some data are available for Anisakis s.l., (i.e. Vidaček, et al, 2010; EFSA 2010) there have been no systematic studies of the thermal effect on different Anisakis species including data related to the habitat, fish species infested and storage parameters before applying the thermal treatment. Furthermore, there is much less information available on the resistance of other parasites to time temperature conditions. It is important to define

with precision at which point the parasites are no longer viable/infective since a too short treatment may lead to health problems, but too extensive treatments may cause substantial economic losses in the industry as well as quality problems for the consumers. This study will be carried out for the fishery products and parasite species of highest interest for the European market. The aim is to better understand, under controlled conditions of the time and temperature applied at the thermal centre of the different products, including different culinary treatments, the effects of these two parameters and find the minimal treatments needed to kill the parasites considered as most relevant in the fishery species of interest. The effectiveness of the treatments will be assayed initially in Anisakis simplex s.s. selecting one geographic area (North Atlantic area) and one species: hake (Merluccius merluccius). Once the effective conditions for the most successful treatments are fixed they will be assayed in isolated larvae of different species and geographic area (Mediterranean Sea), model systems of fishery products artificially parasitized, and in commercial fish.

7.3.2. Study of the effectiveness of other physical and chemical treatments: Well-known (heat: conventional and microwaves, freezing, salt and acid treatments, etc.) and also new emergent food technologies like high hydrostatic pressure, low voltage current, modified atmosphere packaging, electrolyzed-oxidizing water, radiofrequency, ultrasonic waves will be evaluated. At present there has been little work on isolated Anisakis s.p. or infected fish using some of the cited techniques. As in 7.3.1. the effectiveness of the treatments with these novel techniques will be evaluated initially in Anisakis simplex (s.s), selecting one habitat (North Atlantic area) and one species: hake (Merluccius merluccius) in order to fix the optimal conditions to kill the larvae and cause minimal changes in fish muscle. Similarly, some natural products (or their extracts) with anti-parasitic properties will be evaluated in isolated larvae of different species and habitat (Mediterrean Sea), model systems of fishery products artificially parasitized, and commercial fish. The treatments will be applied individually as well as in combination.

7.3.3. Assessment of the quality of the fish product after selected treatments: Monitoring the changes occurring in the fish matrix will be carried out by analysing fish muscle subjected to the successful treatments from 7.3.1 and 7.3.2. This will lead to optimization of the conditions in terms of quality and safety of the product. Vibrational spectroscopy (Fourier Transform (FT)-infrared and FT-Raman) (Careche et al. 1999; Herrero et al. 2004,2005; Sanchez-Alonso et al. 2011), Low Field Nuclear Magnetic Resonance (LF-NMR) (Sanchez-Alonso et al. 2011) and standard physical chemical procedures for quality of the fish muscle and sensory analysis will be used.

7.3.4. Tools to verify that the fishery products have been subjected to a given physical or chemical treatment sufficient to kill the parasite: Collection of LF NMR, Raman and infrared spectra from task 7.2.3 will allow us to search for markers associated with changes undergone by the fish matrix after the most successful alternative treatments have been given. These changes should not be sufficient to impair the quality of the product but it is expected that they will be big enough to confirm that the fish muscle has been subject to a given treatment. Thus, these markers will be used to design a tool to verify this process authentication. In particular, procedures based on LF NMR spectroscopy will be developed as a tool to verify whether the fish has been previously frozen according to the current regulation.

Task 7.4 Development of antigen elimination or inactivation methods

Task Leader: CSIC (ICTAN)

Participants: SERMAS

Application of specific treatments to reduce or inactivate the allergenic capacity: Based on the structural and physical-chemical features of the different allergens, it is possible to elaborate a strategy to eliminate the allergens (e.g. selective precipitations) or reduce their activity (e.g. by crosslinking to other proteins present such as in industrial processes used to elaborate surimi gels). These protocols will be first assayed in model systems and then applied over parasitized muscle systems detecting the retained antigenicity of the treated samples compared with untreated ones. Then, efficiency of the selected treatments for inactivating or reducing the allergenic capacity will be assessed by immunoblotting using sensitized-patient's sera and the quality of the final products will be analysed by physical-chemical methods specifically targeted to each matrix or product.

Task 7.5 Technological enhancement of a tech device to kill zoonotic Anisakids in offal

Task leader: CSIC (IIM-E)

Participants: CSIC (ICTAN), CETMAR, ARVI

Design and development of a prototype to be implemented on the deck of a fishing vessel: The aim of this application is to design and develop a prototype to kill any anisakid present in fish offal discarded at sea after regular post-catch processing on-board the vessels. We will concentrate on the improvement of the design and development of an on-board tech-device (SPINBOARD, Sea Parasite Inactivation On Board) to be implemented

in the deck of a fishing vessel operating in the Grand Sole area. To that end, it will be necessary to design the electronic schemes and modules, modelling with Multisim, CAD design for electromagnetic radiation, manufacturing a complete prototype including specific interface and software, elaborate manual and tech guides, obtaining the EC Label (AT4 Wireless), installation of the equipment on-board, and finally checking the functioning of the device with new trials on-board. This will be done during two fishing seasons at Grand Sole. To that end, a technician will go on board to check the device, to collect the viscera for parasite inspection in the laboratory and to obtain valuable information on the operations on the deck related to the Spinboard. The accuracy and efficiency of Spinboard as a technological solution for management of parasite contaminants in residues during the gutting and discarding operations in the vessel fleet under animal by-product Regulations 1069/2009 and 142/2011 will be the output of the task.

### Participant Role

CSIC (ICTAN) WP leadership. Application of technological treatments, fish quality monitoring, development of methods to verify the given technological solutions applied to kill parasites, mitigation treatments to inactivate allergenic capacity, IHC, SEM. Provision of Parasites via MercaMadrid. Data collection.

CSIC (IIM-E) Data collection. Study viability and infectivity as a function of host fish species. Oral challenges to study passage through different hosts. Development of a prototype to implement in vessels. Study of bacteria-parasite interaction by ESM approach and flesh colonization.

CSIC (MNCN) Taxonomy of Anisakis spp; Analysis of Anisakis spp. viability by ESM and optical microscopy. Study of bacteria-parasite interaction by ESEM approach and flesh colonization.

SERMAS Determination of the antigenicity and allergenicity of fishery products.

ARVI Provision of parasites. Implementation of the device in the vessels and performance of the on board experiments.

UOC Infectivity studies with rodent models

NIFES Studying the effect on shelf-life by establishing interaction models between bacteria and anisakid nematodes in fishery products

CETMAR Management of technical specifications to homologate the prototype to be used on board

UT-URS Genetic identification of samples

ANSES Viability and infectivity of parasites

### Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	CSIC	33.25
2	NIFES	4.00
3	UT-URS	1.00
4	ANSES	1.00
5	CETMAR	3.00
6	SERMAS	2.00
13	UCPH	5.00
17	ARVI	14.00
	Total	63.25

### List of deliverables

Delive- rable Number 61	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature 62	Dissemi- nation level <sup>63</sup>	Delivery date <sup>64</sup>
D7.1	Report on viability and infectivity of parasites in fish and fishery products	1	12.00	R	PU	20
D7.2	Report on bacteria-parasite interaction in fishery products	2	6.00	R	PU	24
D7.3	Report on treatments for killing parasites in fishery products	1	8.00	R	PP	32
D7.4	Report on parasite antigen elimination or inactivation methods	1	8.00	R	PP	32
D7.5	Prototype for management on board of parasite contaminants	1	12.00	Р	PP	32
D7.6	Guideline for parasite risk management in the food chain	1	6.00	R	PU	36
	A	Total	52.00			<u>.</u>

#### Description of deliverables

D7.1) Report on viability and infectivity of parasites in fish and fishery products: Report on viability and infectivity of parasites in fish and fishery products in relation to host species, and effects on passage through different hosts [month 20]

D7.2) Report on bacteria-parasite interaction in fishery products: Report on bacteria-parasite interaction in fishery products [month 24]

D7.3) Report on treatments for killing parasites in fishery products: Report on treatments for killing parasites in fishery products [month 32]

D7.4) Report on parasite antigen elimination or inactivation methods: Report on parasite antigen elimination or inactivation methods in fishery products [month 32]

D7.5) Prototype for management on board of parasite contaminants: Prototype for management on board of parasite contaminants in fish residues [month 32]

D7.6) Guideline for parasite risk management in the food chain: Guideline for parasite risk management in the food chain [month 36]

### Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead benefi- ciary number	Delivery date from Annex I <sup>60</sup>	Comments
MS11	Data on food-chain interventions to reduce the parasite risk	1	30	
MS12	Start of field trials for the parasite killing prototype on-board	1	33	

Project Number <sup>1</sup>	r <sup>1</sup> 312068		Project Acronym <sup>2</sup>	Р	ARASITE
		One form per Work Pacl	kage	9	
Work package number	r <sup>53</sup>	WP8	Type of activity <sup>54</sup>		RTD
Work package title		QUANTITATI	VE RISK ANALYSIS		
Start month		1			
End month		36			
Lead beneficiary numb	oer 55	15			

#### Objectives

1. Summarize, analyze and predict consumer exposure to fish parasites through statistical modelling of patterns and trends in the incidence of zoonotic parasites in commercially important fish species (and in seafood products.

2. Develop a dynamic framework to integrate parasite infestation estimates and genetic variability estimates, as a means to predict parasite abundance.

3. Collate and analyze available data on the incidence of parasite infections in humans to build up a general picture of the geographical and temporal patterns and identify hotspots.

4. Undertake a quantitative risk assessment, with a particular focus on the probability of illness from parasites associated with the consumption of raw or partially cooked seafood products and estimating the effectiveness of proposed risk mitigation strategies.

5. Model consumer willingness to pay for treatments to reduce the incidence of parasites in fish products.

6. Define and evaluate Cost/Benefit scenarios on the application of treatments and tools for policymakers and food producers.

### Description of work and role of partners

Task 8.1 Statistical modelling and inference

8.1.1. Data collation, screening, exploration and mapping

Task Leader: UNIABDN

Participants: UT-URS, NIFES, CSIC (IIM-E)

Data sets on parasite numbers in seafood will be provided through outputs of WPs 2 and 4, focussing on the major commercial seafood species and most important parasites. The seafood species considered will thus include herring, anchovy, mackerel, blue whiting, bluefin tuna, scabbardfish, European hake, haddock, cod, monkfish, and sea bass, as well as the imported case study species Nile perch, Sutchi catfish, Thunnus spp. and Todarodes pacificus.

Data on parasites will mainly concern Anisakis spp. (mainly A. simplex and A. pegreffii), extending to other species such as Pseudoterranova decipiens s.l. and Contraceacum osculatum s.l. where adequate data are available.

All data sets will be screened for errors and where necessary data will be checked with the data source. In addition to data on individual fish sampled we expect to generate aggregated data at appropriate spatial and temporal scales (e.g. by ICES subdivision (or equivalent for the Mediterranean) and season, although with a finer resolution where data availability permits). Basic and model-based distribution maps will be generated using Geographic Information Systems (GIS) to complement those generated in other WPs. As soon as sufficient data are available, power analyses will be carried out to check whether sampling levels used in WP2 could be reduced without loss of statistical power

8.1.2. Develop statistical models for fish parasite distribution and abundance in commercial seafood species from European fishing grounds and marketed in fishery products.

Task Leader: UNIABDN

Participants: NIFES, UT-URS

Parasite prevalence will be modelled for those combinations of seafood species, parasite species, areas and time periods best represented in the project database. This will include models at the level of individual fish to identify size-, season and geographic patterns as well as broader-scale models based on aggregated data.

Statistical modelling will use a Generalised Linear Mixed effects Modelling (GLMM) framework, with extensions to smoothing techniques (generalised additive mixed effects modelling) and allowing for zero inflation and spatial and temporal correlation (Zuur et al., 2012), allowing us to deal with a relatively complex combination of non-linear effects and interactions of a several explanatory variables. Data exploration, model fitting and model validation will follow standard procedures (see Zuur et al. 2007, 2009, 2010) and will use R software and Markov Chain Monte Carlo techniques using winBUGS. Sub-sets of data will be held back for use in testing model predictions. An additional model validation stage is the successful prediction of hotspots of parasite-related human health issues.

8.1.3. Develop a dynamic framework to integrate parasite infestation estimates and genetic variability estimates. Task Leader: UNIABDN

### Participants: UT-URS

The relationship between parasitic infestation levels and genetic variability estimates in different species/populations of anisakids will be modelled based on a similar framework to 8.1.2 although restricted to some case studies (i.e. Anisakis pegreffii and A. simplex s.s). For the selected species and populations of these parasites, estimates of genetic diversity, gene diversity and haplotype diversity will be derived from different sets of genetic data obtained in WP4 based on nuclear (allozymes, microsatellites) and mitochondrial markers. The correlations between parasitic infestation levels observed in hosts populations and genetic variability estimates of the anisakids will be quantified. Coupled with results from 8.1.2, this will potentially allow predictions to be made about parasite prevalence and density in host fish species from understudied areas.

Task 8.2 Evaluate prevalence of human health impacts of parasites in seafood

Task Leader: UNIABDN

### Participants: UT-URS, SERMAS

Some project participants are involved in the diagnostic parasitological analysis by the main hospitals in Spain involved in the project, and have described in the past the occurrence of several cases of human anisakiasis. This activity will continue during the implementation of the project proposal leading to the reporting of other cases of human anisakiasis and relating those cases with seafood consumption. In addition, national and European health service laboratories holding data on incidence of anisakiasis and related parasite-related incidents will be surveyed (e.g., the European Centre for Disease prevention and control, the UK-NHS, the Policlinico Umberto I Hospital in Rome, Pescara/Benevento/Viterbo Hospitals,...). This WP will also collate data held by partners and published through national/regional health statistical reports and the scientific literature, to build up a picture of seafood parasite-related human health impacts in Europe over the last two decades.

Task 8.3 Quantitative risk assessment

### Task Leader: UNIABDN

### Participants: CETMAR

The risk assessment requires an initial statement of purpose and the process involves four primary stages: (1) hazard identification, which identifies the pathogenic organism of concern and whether it is actually a hazard in the context that it is being studied; (2) exposure assessment, to determine the number of organisms ingested; (3) hazard characterization, which gives a quantitative or qualitative assessment of the adverse effects of the pathogen to humans; more specifically a dose-response model can be implemented which mathematically models the variability in impact (response) following exposure to different doses (McNab, 1997); (4) risk characterization, which gives a probability of occurrence of the illness and also the severity of the health effects in a given population.

A process based risk assessment model will be developed using @RISK software. The model will include variability and uncertainty in input data and simulation will be carried out using the Monte Carlo technique. The model will be parameterised for two areas within the EU, one with high (Spain) and one with low (UK) levels of human disease incidence. The initial model will concentrate on anisakid but other parasites will be included where data permits. Fish and shellfish species that will be included are those that are typically eaten raw or minimally processed in these two countries. Also, fish that are imported from outside of the EU will be included.

(a) Prevalence and load of parasites in fish flesh.

This will use data from WP2 on prevalence of parasites from key commercial

species (EU production and imports from Asia) as well as data from the literature

(See EFSA www.efsa.europa.eu/en/efsajournal/doc/1543.pdf and FSAS

www.foodbase.org.uk//admintools/reportdocuments/306-1-618\_Final\_Report\_S14008\_Anisakis.pdf reports) (b) Storage and treatment.

This considers the efficacy of detecting and removing parasites from fish (including visual, high hydrostatic pressure, modified atmospheric packing. Efficacy of methods for killing parasites.

### (c) Consumption

This concerns consumption of raw fish across the whole population and in high risk groups in both Spain and the UK. A questionnaire will be designed that will collate information on frequency and pathway of exposure. This will be carried out by telephone across a random sample of the population (1000 respondents). A further survey (n=200) will be conducted in a high risk group including individuals that have been infected (where possible). This will be done by interview, following the relevant informed consent, confidentiality and data prospection requirements. These data will elucidate the infection pathways and the risky behaviour of the respondents. (d) Dose response

In Microbiological risk assessment this is conventionally generated using data from outbreaks or from volunteer studies or animal model studies. These data do not exist for these parasites. The approach that will be used will require utilising data on ingested dose from parasites contained within fish together with amount of fish consumed per person across a population. To then fit this to the human epidemiological data (From 8.2) across the population using conventional dose response models (exponential and beta-Poisson). Note this is not an ideal solution but will enable evaluation of risk mitigation strategies to be carried out. (e) Illness.

It will be possible to simulate the size of sensitive populations and also subsequent risk of allergic shock. This can then be compared with epidemiological findings.

A sensitivity analysis will be performed to identify the key variables that have the strongest effect on the risk estimates.

The different risk mitigation strategies identified within the Project will be evaluated for the two study areas. The likely effect of their implementation in terms of reducing human disease will be determined.

Task 8.4. Analysis of willingness to pay

Task Leader: UNIABDN

Participants: CETMAR, LARPRO

This task includes design the scenarios and the content of the questionnaires suitable for the analysis of consumer preferences for fishery products treated with different methods of parasite elimination. The creation of vignettes involves four steps:

Step 1: Identifying the characteristics - The characteristics or attributes of a fishery product are identified (e.g. price, quality of flesh, level of health impact etc.).

Step 2: Assigning levels to the characteristics - The levels must be plausible and actionable, thus encouraging the respondents to take the exercise seriously.

Step 3: Design of scenarios (vignettes) - Scenarios are drawn up that describe all possible fishery product configurations, given the selected attributes and level possibilities. Since the number of scenarios increases with the number of characteristics and levels, not all of the scenarios generated can be included in the questionnaire as the respondents have a finite attention span. Thus, experimental designs are used to reduce the number to a convenient level.

Step 4: Establishing preferences - Once designed, the vignettes are offered to respondents, who are asked to state their preferences. Preferences for the scenarios included in the questionnaire are elicited by using a ranking method.

The new data on consumer preferences will be derived from a survey of consumers. We envisage 500-800 individuals to be surveyed (quota survey) in each participant country for the generation of the data set to be used for the statistical analysis, which will follow a multinomial regression-based methodology.

The collation and analysis of newly obtained data consistently across several European countries (including the UK and Spain as in 8.3 but also extending to other partner countries) on consumer preference for such products will provide new opportunities to identify major factors that affect individual consumer demand.

Task 8.5 Cost/Benefit scenarios policy makers/food producers

Task Leader: LARPRO

Participants: UNIABDN

Description of work.

Based on the different methods of parasite elimination and risk assessment developed, two general types of cost/benefit scenarios will be further defined and evaluated:

- Policy change scenarios: cost/benefit evaluation of potential changes of policy for border inspection

- Value chain scenarios: cost/benefit evaluations on potential changes of inspection/control method at food producer (fishery products transformation)

In each scenario the applicable parasite elimination method or risk evaluation tool will be analysed with regard to its degree of applicability and effectiveness, vs. cost, comparing to tools and methods used currently.

For the Policy change scenarios, 2-3 border control authorities will be invited to participate, in order to verify the data used for the definition and evaluation. For the Value chain scenarios, 2-3 food producers (fishery products transformation) will be invited to participate, in order to verify the data used for the definition and evaluation.

### Participant Role

UNIABDN WP leadership, statistical analysis, WTP analysis

UT-URS Data collection, correlation between anisakid abundance and genetic variability values

NIFES Data collection and exploration, basic statistical modelling

CSC (IIM-E) Data collection and mapping, quantitative risk assessment, analysis of willingness to pay

CETMAR Quantitative risk assessment, analysis of willingness to pay

SERMAS Prevalence of human health impacts of parasites in seafood

LARPRO Cost-benefit analysis Policy makers and Food producers

### Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	CSIC	1.50
2	NIFES	3.00
3	UT-URS	3.00
5	CETMAR	4.00
6	SERMAS	2.00
15	UNIABDN	22.00
16	LARPRO	13.00
	Total	48.50

### List of deliverables

Delive- rable Number	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature 62	Dissemi- nation level <sup>63</sup>	Delivery date <sup>64</sup>
D8.1	Report on statistical analysis of parasite abundance and genetic variability.	15	6.00	R	PU	28
D8.2	Report on quantitative risk assessment.	15	18.00	R	PU	36
D8.3	Report on Willingness to Pay.	15	9.00	R	PU	36
D8.4	Report on Cost/Benefit scenarios Policy/Food producers.	16	15.00	R	PU	36
		Total	48.00			

Description of deliverables

D8.1) Report on statistical analysis of parasite abundance and genetic variability.: Report on statistical analysis of parasite abundance and genetic variability. [month 28]

D8.2) Report on quantitative risk assessment.: Report on quantitative risk assessment. [month 36]

D8.3) Report on Willingness to Pay.: Report on Willingness to Pay. [month 36]

D8.4) Report on Cost/Benefit scenarios Policy/Food producers.: Report on Cost/Benefit scenarios Policy/Food producers. [month 36]

	Schedule of relevant Milestones							
Milestone number <sup>59</sup>	Milestone name	Lead benefi- ciary number	Delivery date from Annex I <sup>60</sup>	Comments				
MS13	Completed exposure survey.	15	24					
MS14	Design of data analysis protocols.	15	28					
MS15	Completed risk analysis (including willingness to pay and cost-benefit scenarios)	15	32					

Project Number <sup>1</sup> 312068		F	Project Acronym <sup>2</sup>	PA	ARASITE	
				form per Work Packa	ige	
Work package number	5 <sup>3</sup>	WP9	Тур	be of activity <sup>54</sup>		OTHER
Work package title		INNOVATION, COMMUNICATION AND DISSEMINATION ACTIVITIES				ISSEMINATION ACTIVITIES
Start month		1				
End month		36				
Lead beneficiary numb	ber 55	5				

#### Objectives

1. To achieve effectiveness in communicating the risks associated with fish/seafood parasites targeted in this project, increasing transparency, enhancing the benefits of fish consumption and explaining the measures and possible paths and tools to tackle such risks.

2. 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.

3. To spread the knowledge achieved and disseminate results to de different stakes holders: scientific bodies, policy-makers at European, national and regional level, to the industry and to consumers and civil society.

### Description of work and role of partners

For the European Commission, the value added by the topic PARASITE proposal addresses, relies on offering safe and high-quality seafood to consumers as well as in strengthening the competitiveness of the European food producers and a strong participation of SMEs is needed to contribute to that benefit.

These expectations about the project impact make it necessary to implement a common strategy comprising knowledge transfer on one hand and dissemination on the other hand. The reason to formulate one single strategy focusing on both aspects to be developed in parallel has to do with the potential of technology and knowledge transfer to provide pathways to the right operators and upgrade their capacity to address the risks associated to fish and seafood parasites. This can be managed as an opportunity for the European seafood industry to strengthen its competitiveness. A good dissemination and bi-directional communication strategy will be a key element to guarantee on one hand an effective and accurate transmission of information to seafood markets and to increase trust and confidence in these markets among European seafood producers, processors and retailers.

This workpackage comprises a total of six tasks, three of them focusing on technology and knowledge transfer and the other three more related to dissemination and communication:

Task 9.1. Catalogue of technological results obtained within project and assessment on its market potential Task Leader: CETMAR

Participants: CSIC (IIM-E), NIFES, UT-URS, CHG3, ARVI, TECNET, UNIABDN Description of work.

The catalogue must comprise a description of project results aiming to be comprehensive, clear and useful for the most relevant end users (mainly from the industry but also for other potential end-users of the project results).

This will require in each case the characterisation of the main end-user's groups and segments; the identification of needs and barriers for technological absorption; and the main pros and constraints for the implementation of the technologies included in the portfolio.

The structure of the catalogue will be proposed by CETMAR and discussed with partners during 1 month. Partners responsible for each transferable result will get involved in the process to prepare the contents of the catalogue regarding such results. End-users represented within the consortium will be consulted about the quality and interest of the information provided, and their considerations will be used to prepare a final version available for any interested end user. In all cases, interests regarding IPR will be carefully observed.

Task 9.2. Tech transfer support by reinforcing end users' skills.

Task Leader: CETMAR

Participants: CSIC (IIM-E), NIFES, UT-URS, CHG3, ARVI, TECNET, SERMAS, CSIC (ICTAN), NEDERLOF'S, HERMES, UNIABDN

Description of work.

This will consist in two main kind of actions:

Training workshops. It is expected that at least three training workshops targeting the industry, consumer organisations and inspection bodies are organised:

- 2 Workshops for the use of diagnostic tools. One for UV and one for RT-PCR once ring trials and beta testing activities have been accomplished (Wp2)

- Workshop on operating strategies and technologies for the fishing fleets and fish processors. (2 workshops on treatments to mitigate the impact of fish and seafood parasites)

Training materials will be produced in electronic multimedia formats (making special use of video recordings) aiming to remain available to be reproduced in different geographic areas by different project partners during and after the project accomplishment.

• Short-term stages of industry and administration staff (potential end-users) in RTD facilities to better understand the technologies being developed and/or used with interest to be transferred. This mechanism will also be used to facilitate the exchange of experiences with Asiatic partners.

RTD partners involved in developing detection, characterisation and treatment tools and practice guidelines for risk management (WP6 and WP8) will provide support to prepare the didactic materials. SMEs involved in beta testing activities will also contribute by disseminating their experience, lessons learnt and best practices. CETMAR will get in charge of compiling and editing the didactic materials, of organising the workshops and of hosting at least two of them.

Task 9.3. 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).

Task Leader: CETMAR

Participants: CSIC (IIM-E), NIFES, UT-URS, CHG3, ARVI, TECNET, SERMAS, CSIC (ICTAN), NEDERLOF'S, HERMES, UNIABDN, ISS

CETMAR will get in charge of this task. On-line questionnaires and webinnar system will be implemented to gather inputs from all partners on discussion documents and hypothesis. Two last project workshops will be used to discuss on interim and final conclusions before delivering the final outcome.

Task 9.4. Analysis of dissemination opportunities and needs

Task leader: CETMAR

Participants: all partners

Analysis of dissemination opportunities and needs and identification and characterisation of the main targets and code for an effective craft of messages and selection of means for transmission. Introduction of the resulting conclusions for the implementation of the dissemination strategy for the project and its results.

Cetmar will propose to project partners an open questionnaire to be answered by them and circulated to a relevant number of stakeholders (at least 100 per country) in each of the countries involved the proposal. By processing this information, CETMAR will obtain a list of recommendations to consider in order improving the dissemination effectiveness.

Task. 9.5. Work with the media professionals

Task Leader: CETMAR

Participants: all partners

Identification of key media, target professionals, and work with them to become a reference source and assure the right orientation of messages

It will be organised a discussion panel with 10 representatives of relevant media and communication agencies (EFE-Agro...) of European and national relevance, to get conclusions on how to collaborate with press professionals in disseminating the project results with guarantees of avoiding any unjustified damage to the industry and improving the accuracy and quality of the information that will reach a wider public in civil society.

Task 9.6. Implementation of communication and dissemination plan Task Leader: CETMAR

Participants: all partners

It will be implemented by means of:

- The project website (can be a basic element to communicate general information about the project) and a channel that can be organised in sections for different targets and opening opportunities for interaction with them.

- Accomplishment of press-conferences, interviews and articles.

- Scientific publications and events.

Two major dissemination events will be added to the above: matching with the second and third editions of the International Symposium Seafood Parasites' Management Strategies, it will be organised a project launch presentation during the first year of the project. For the third edition of the Symposium, the PARASITE project will get more directly involved in the Symposium organisation and results obtained will be offered on presentations, posters and other dissemination support materials (video, leaflets, results' catalogue etc)

### Role

CETMAR Coordinate WP activities and participate in all tasks. Explain the methodology being followed for each task. Compile inputs from the other partners involved in this WP

RTDs All the RTD partners will contribute to this WP. Those with more direct responsibility for transferable results and training workshops will provide key information on the characteristics of such results that make them relevant for the different end users.

Partner SMEs All SMEs will contribute to this WP in three different ways:

1. Providing feed-back as potential end-users of project results to orient them to impact the market.

2. Sharing their own experience in the project with other SMEs (those involved in beta testing and real life trials of project results)

3. Spreading their own knowledge and disseminating the project through their most relevant channels to the seafood industry and, mainly to consumers.

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
1	CSIC	8.60
2	NIFES	3.00
3	UT-URS	3.00
4	ANSES	0.60
5	CETMAR	23.00
6	SERMAS	3.00
7	FAMRI	0.60
8	ISS	2.00
9	IBE	0.60
10	ZOUC	0.60
11	CLSU	0.60
12	MRI	0.60
13	UCPH	0.60
14	IZOR	0.60
15	UNIABDN	3.00
16	LARPRO	1.50
17	ARVI	1.50

### Person-Months per Participant

### Person-Months per Participant

Participant number <sup>10</sup>	Participant short name <sup>11</sup>	Person-months per participant
18	CHG3	1.50
19	TNET	1.50
20	HERMES	0.60
21	NEDERLOF'S	0.60
	Total	57.60

### List of deliverables

Delive- rable Number	Deliverable Title	Lead benefi- ciary number	Estimated indicative person- months	Nature 62	Dissemi- nation level <sup>63</sup>	Delivery date 64
D9.1	Project website	5	3.00	0	PU	5
D9.2	Didactic materials for training workshops	5	4.00	R	RE	30
D9.3	Portfolio of technologies and analysis of market potential	5	6.00	R	RE	36
D9.4	Annual compilation and analysis of PARASITE's media impact.	5	1.00	R	PU	13
D9.5	Annual compilation and analysis of PARASITE's media impact.	5	1.00	R	PU	25
D9.6	Annual compilation and analysis of PARASITE's media impact.	5	1.00	R	PU	36
D9.7	After project action plan (roadmap)	5	3.00	R	PU	36
	~	Total	19.00			~J

### Description of deliverables

D9.1) Project website: Project website [month 5]

D9.2) Didactic materials for training workshops: Didactic materials for training workshops [month 30]

D9.3) Portfolio of technologies and analysis of market potential: Portfolio of technologies and analysis of market potential [month 36]

D9.4) Annual compilation and analysis of PARASITE's media impact.: Annual compilation and analysis of PARASITE's media impact. [month 13]

D9.5) Annual compilation and analysis of PARASITE's media impact.: Annual compilation and analysis of PARASITE's media impact. [month 25]

D9.6) Annual compilation and analysis of PARASITE's media impact.: Annual compilation and analysis of PARASITE's media impact. [month 36]

D9.7) After project action plan (roadmap): After project action plan (roadmap) [month 36]

### Schedule of relevant Milestones

Milestone number <sup>59</sup>	Milestone name	Lead benefi- ciary number	Delivery date from Annex I <sup>60</sup>	Comments
MS16	Public release of project website	5	3	
MS17	Accomplishment of expert panel with media	5	6	
MS18	Accomplishment of first training workshop	5	13	
MS19	Agreement on results catalogue structure.	5	19	
MS20	Accomplishment of 3rd Symposium on Fish Parasite Management Strategies	5	34	

### WT4: List of Milestones

Project Nu	Project Number <sup>1</sup> 312068			Project Acronym <sup>2</sup>			
			List	and S	chedule of Milest	ones	
Milestone number 59	Milestone	name	WP number 53		Lead benefi- ciary number	Delivery date from Annex I 60	Comments
MS1	Implement of the inter manageme platform.	nal	WP1		1	3	
MS2	Complete epidemiological data sets from European wild catch fisheries, case studies on fish imports		WP2		2	24	
MS3	Database ready for management of parasitized fish stocks and products		WP3		18	3	
MS4	Biobank structure and infrastructure		WP3		18	6	
MS5	Internal seminar for providing skills to project Biobank users		WP3		18	7	
MS6	Definition of DNA primers and probes to be used in RT-PCR to detect DNA of different species of anis		WP4		3	16	
MS7	Characterization of the risk exposure to allergens in fishery products with no alive parasites		WP5		6	26	
MS8	Characterization of the mechanisms of allergic sensitisation and exposure to Anisakids		WP5		6	36	
MS9	Proteomics identification of differentially expressed relevant antigens		WP5		6	36	
MS10	Improved methods re harmoniza		WP6		8	26	

### WT4: List of Milestones

Milestone number <sup>59</sup>	Milestone name	WP number 53	Lead benefi- ciary number	Delivery date from Annex I 60	Comments
MS11	Data on food-chain interventions to reduce the parasite risk	WP7	1	30	
MS12	Start of field trials for the parasite killing prototype on-board	WP7	1	33	
MS13	Completed exposure survey.	WP8	15	24	
MS14	Design of data analysis protocols.	WP8	15	28	
MS15	Completed risk analysis (including willingness to pay and cost-benefit scenarios)	WP8	15	32	
MS16	Public release of project website	WP9	5	3	
MS17	Accomplishment of expert panel with media	WP9	5	6	
MS18	Accomplishment of first training workshop	WP9	5	13	
MS19	Agreement on results catalogue structure.	WP9	5	19	
MS20	Accomplishment of 3rd Symposium on Fish Parasite Management Strategies	WP9	5	34	

# WT5: Tentative schedule of Project Reviews

Project Nu	mber <sup>1</sup>	312068 Project A		ronym <sup>2</sup>	PARASITE		
Tentative schedule of Project Reviews							
Review number <sup>65</sup>	Tentative timing	Planned venue of review		Comments	, if any		
RV 1	21	BRUSSELS		MID-TERM PROJECT REVIEW			

WT6: Project Effort by Beneficiary and Work Package

Project Number <sup>1</sup>	312	312068			Project Acronym <sup>2</sup>			PARASITE			
		Indica	ative efforts	(man-mon	ths) per Be	neficiary p	er Work Pa	ckage			
Beneficiary number and short-name	WP 1	WP 2	WP 3	WP 4	WP 5	WP 6	WP 7	WP 8	WP 9	Total per Beneficiary	
1 - CSIC	6.00	9.50	14.00	5.00	22.25	14.00	33.25	1.50	8.60	114.10	
2 - NIFES	0.50	9.00	3.00	0.00	0.00	4.00	4.00	3.00	3.00	26.50	
3 - UT-URS	0.50	8.00	6.00	16.00	0.00	5.00	) 1.00	3.00	3.00	42.50	
4 - ANSES	0.50	8.00	0.00	7.00	0.00	4.00	) 1.00	0.00	0.60	21.10	
5 - CETMAR	0.50	0.00	0.00	0.00	0.00	5.00	) 3.00	4.00	23.00	35.50	
6 - SERMAS	0.50	0.00	3.00	0.00	20.00	0.00	2.00	2.00	3.00	30.50	
7 - FAMRI	0.50	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.60	5.10	
8 - ISS	0.50	0.00	0.00	0.00	4.00	4.00	0.00	0.00	2.00	10.50	
9 - IBE	0.50	15.00	0.00	6.00	0.00	0.00	0.00	0.00	0.60	22.10	
10 - ZOUC	0.50	15.00	0.00	16.00	0.00	0.00	0.00	0.00	0.60	32.10	
11 - CLSU	0.50	15.00	0.00	16.00	0.00	0.00	0.00	0.00	0.60	32.10	
12 - MRI	0.50	14.00	0.00	0.00	0.00	4.00	0.00	0.00	0.60	19.10	
13 - UCPH	0.50	3.00	0.00	0.00	0.00	0.00	) 5.00	0.00	0.60	9.10	
14 - IZOR	0.50	6.00	0.00	9.00	0.00	0.00	0.00	0.00	0.60	16.10	
15 - UNIABDN	0.50	6.00	0.00	0.00	0.00	0.00	0.00	22.00	3.00	31.50	
16 - LARPRO	9.00	0.00	0.00	0.00	0.00	0.00	0.00	13.00	1.50	23.50	
17 - ARVI	0.50	12.00	3.00	0.00	0.00	7.00	) 14.00	0.00	1.50	38.00	
18 - CHG3	0.50	0.00	18.00	0.00	0.00	6.00	0.00	0.00	1.50	26.00	
19 - TNET	0.50	0.00	0.00	0.00	0.00	16.00	0.00	0.00	1.50	18.00	
20 - HERMES	0.50	12.00	0.00	0.00	0.00	6.00	0.00	0.00	0.60	19.10	
21 - NEDERLOF'S	0.50	12.00	0.00	0.00	0.00	6.00	0.00	0.00	0.60	19.10	
Total	24.50	148.50	47.00	75.00	46.25	81.00	63.25	48.50	57.60	591.60	

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WT7: Project Effort by Activity type per Beneficiary

Project Number <sup>1</sup>		312068			Proje	ct Acronym	2	PAI	RASITE					
	Indicative efforts per Activity Type per Beneficiary													
Activity type	Part. 1 CSIC	Part. 2 NIFES	Part. 3 UT-URS	Part. 4 ANSES	Part. 5 CETMAR	Part. 6 SERMAS	Part. 7 FAMRI	Part. 8 ISS	Part. 9 IBE	Part. 10 ZOUC	Part. 11 CLSU	Part. 12 MRI	Part. 13 UCPH	Part. 14 IZOR
1. RTD/Innovation activities														
WP 2	9.50	9.00	8.00	8.00	0.00	0.00	4.00	0.00	15.00	15.00	15.00	14.00	3.00	6.00
WP 3	14.00	3.00	6.00	0.00	0.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 4	5.00	0.00	16.00	7.00	0.00	0.00	0.00	0.00	6.00	16.00	16.00	0.00	0.00	9.00
WP 5	22.25	0.00	0.00	0.00	0.00	20.00	0.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00
WP 6	14.00	4.00	5.00	4.00	5.00	0.00	0.00	4.00	0.00	0.00	0.00	4.00	0.00	0.00
WP 7	33.25	4.00	1.00	1.00	3.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00	0.00
WP 8	1.50	3.00	3.00	0.00	4.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Research	99.50	23.00	39.00	20.00	12.00	27.00	4.00	8.00	21.00	31.00	31.00	18.00	8.00	15.00
2. Demonstration ac	tivities													
Total Demo	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3. Consortium Mana	- 				r							r		
WP 1	6.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total Management	6.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
4. Other activities	4. Other activities													
WP 9	8.60	3.00	3.00	0.60	23.00	3.00	0.60	2.00	0.60	0.60	0.60	0.60	0.60	0.60
Total other	8.60	3.00	3.00	0.60	23.00	3.00	0.60	2.00	0.60	0.60	0.60	0.60	0.60	0.60
Total	114.10	26.50	42.50	21.10	35.50	30.50	5.10	10.50	22.10	32.10	32.10	19.10	9.10	16.10

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WT7: Project Effort by Activity type per Beneficiary

			•	•	•		•
Part. 15 UNIABDN	Part. 16 LARPRO	Part. 17 ARVI	Part. 18 CHG3	Part. 19 TNET	Part. 20 HERMES	Part. 21 NEDERLO	Total
6.00	0.00	12.00	0.00	0.00	12.00	12.00	148.50
0.00	0.00	3.00	18.00	0.00	0.00	0.00	47.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00	75.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00	46.25
0.00	0.00	7.00	6.00	16.00	6.00	6.00	81.00
0.00	0.00	14.00	0.00	0.00	0.00	0.00	63.25
22.00	13.00	0.00	0.00	0.00	0.00	0.00	48.50
28.00	13.00	36.00	24.00	16.00	18.00	18.00	509.50
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ivities							
0.50	9.00	0.50	0.50	0.50	0.50	0.50	24.50
0.50	9.00	0.50	0.50	0.50	0.50	0.50	24.50
rr	T						
3.00	1.50	1.50	1.50	1.50	0.60	0.60	57.60
3.00	1.50	1.50	1.50	1.50	0.60	0.60	57.60
31 50	23.50	38.00	26.00	18.00	19.10	10 10	591.60
	UNIABDN 6.00 0.00 0.00 0.00 0.00 0.00 0.00 0.0	UNIABDN         LARPRO           6.00         0.00           0.00         0.00           0.00         0.00           0.00         0.00           0.00         0.00           0.00         0.00           0.00         0.00           0.00         0.00           22.00         13.00           28.00         13.00           0.00         0.00           0.00         0.00           0.00         0.00           0.00         0.00           3.00         1.50           3.00         1.50	UNIABDN         LARPRO         ARVI           6.00         0.00         12.00           0.00         0.00         3.00           0.00         0.00         0.00           0.00         0.00         0.00           0.00         0.00         0.00           0.00         0.00         0.00           0.00         0.00         14.00           0.00         0.00         14.00           22.00         13.00         0.00           28.00         13.00         36.00           0.00         0.00         0.00           0.50         9.00         0.50           0.50         9.00         0.50           3.00         1.50         1.50	UNIABDN         LARPRO         ARVI         CHG3           6.00         0.00         12.00         0.00           0.00         0.00         3.00         18.00           0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00           0.00         0.00         14.00         0.00           0.00         0.00         14.00         0.00           22.00         13.00         36.00         24.00           28.00         13.00         0.00         0.00           0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00           0.00         0.00         0.50         0.50           0.50         9.00         0.50         0.50           0.50         1.50         1.50         1.50           3.00         1.50         1.50         1.50 </td <td>UNIABDN         LARPRO         ARVI         CHG3         TNET           6.00         0.00         12.00         0.00         0.00           0.00         0.00         3.00         18.00         0.00           0.00         0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00         0.00           0.00         0.00         7.00         6.00         16.00           0.00         0.00         14.00         0.00         0.00           22.00         13.00         0.00         0.00         0.00           28.00         13.00         36.00         24.00         16.00           0.00         0.00         0.50         0.50         0.50           0.50         9.00         0.50         0.50         0.50           0.50         9.00         0.50         0.50         0.50           0.50         9.00         1.50         1.50         1.50           3.00         1.50         1.50         1.50         1.50</td> <td>UNIABDN         LARPRO         ARVI         CHG3         TNET         HERMES           6.00         0.00         12.00         0.00         0.00         12.00           0.00         0.00         3.00         18.00         0.00         0.00           0.00         0.00         0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00         0.00         0.00           0.00         0.00         7.00         6.00         16.00         6.00           0.00         0.00         7.00         6.00         16.00         6.00           0.00         0.00         7.00         0.00         0.00         0.00           22.00         13.00         0.00         0.00         0.00         0.00           28.00         13.00         36.00         24.00         16.00         18.00           ivities        </td> <td>UNIABDNLARPROARVICHG3TNETHERMESNEDERLO6.000.0012.000.000.0012.0012.000.000.0012.000.007.006.0016.006.006.000.000.0014.000.000.000.000.000.000.0014.000.000.000.000.0022.0013.000.000.000.000.000.0028.0013.0036.0024.0016.0018.0018.00ivitiesivities0.000.000.500.500.500.500.509.000.500.500.500.500.500.509.000.501.501.500.600.603.001.501.501.500.600.603.001.501.501.500.600.60</td>	UNIABDN         LARPRO         ARVI         CHG3         TNET           6.00         0.00         12.00         0.00         0.00           0.00         0.00         3.00         18.00         0.00           0.00         0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00         0.00           0.00         0.00         7.00         6.00         16.00           0.00         0.00         14.00         0.00         0.00           22.00         13.00         0.00         0.00         0.00           28.00         13.00         36.00         24.00         16.00           0.00         0.00         0.50         0.50         0.50           0.50         9.00         0.50         0.50         0.50           0.50         9.00         0.50         0.50         0.50           0.50         9.00         1.50         1.50         1.50           3.00         1.50         1.50         1.50         1.50	UNIABDN         LARPRO         ARVI         CHG3         TNET         HERMES           6.00         0.00         12.00         0.00         0.00         12.00           0.00         0.00         3.00         18.00         0.00         0.00           0.00         0.00         0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00         0.00         0.00           0.00         0.00         0.00         0.00         0.00         0.00           0.00         0.00         7.00         6.00         16.00         6.00           0.00         0.00         7.00         6.00         16.00         6.00           0.00         0.00         7.00         0.00         0.00         0.00           22.00         13.00         0.00         0.00         0.00         0.00           28.00         13.00         36.00         24.00         16.00         18.00           ivities	UNIABDNLARPROARVICHG3TNETHERMESNEDERLO6.000.0012.000.000.0012.0012.000.000.0012.000.007.006.0016.006.006.000.000.0014.000.000.000.000.000.000.0014.000.000.000.000.0022.0013.000.000.000.000.000.0028.0013.0036.0024.0016.0018.0018.00ivitiesivities0.000.000.500.500.500.500.509.000.500.500.500.500.500.509.000.501.501.500.600.603.001.501.501.500.600.603.001.501.501.500.600.60

### WT8: Project Effort and costs

Project Nu	umber <sup>1</sup>	312068		Project Acronym <sup>2</sup> PARASITE						
	Project efforts and costs									
			Estimated							
Benefi- ciary number	ciary Beneficiary	Effort (PM)	Personnel costs (€)	Subcontracting (€)	Other Direct costs (€)	Indirect costs OR lump sum, flat-rate or scale-of-unit (€)	Total costs	Total receipts (€)	Requested EU contribution (€)	
1	CSIC	114.10	355,710.00	24,000.00	140,000.00	535,681.00	1,055,391.00	0.00	825,874.00	
2	NIFES	26.50	132,500.00	0.00	35,000.00	189,275.00	356,775.00	0.00	282,225.00	
3	UT-URS	42.50	127,500.00	0.00	99,500.00	136,200.00	363,200.00	0.00	280,600.00	
4	ANSES	21.10	54,860.00	0.00	78,000.00	79,716.00	212,576.00	0.00	162,176.00	
5	CETMAR	35.50	117,150.00	15,000.00	18,000.00	27,030.00	177,180.00	0.00	163,800.00	
6	SERMAS	30.50	100,650.00	10,000.00	49,000.00	89,790.00	249,440.00	0.00	194,100.00	
7	FAMRI	5.10	15,300.00	0.00	34,000.00	29,580.00	78,880.00	0.00	62,880.00	
8	ISS	10.50	42,000.00	0.00	89,000.00	78,600.00	209,600.00	0.00	163,600.00	
9	IBE	22.10	13,260.00	0.00	19,000.00	19,356.00	51,616.00	0.00	41,376.00	
10	ZOUC	32.10	19,260.00	0.00	28,000.00	9,452.00	56,712.00	0.00	44,532.00	
11	CLSU	32.10	19,260.00	0.00	28,000.00	9,452.00	56,712.00	0.00	44,532.00	
12	MRI	19.10	84,040.00	0.00	53,000.00	82,224.00	219,264.00	0.00	168,784.00	
13	UCPH	9.10	38,220.00	0.00	9,000.00	28,332.00	75,552.00	0.00	59,712.00	
14	IZOR	16.10	27,370.00	0.00	33,300.00	36,402.00	97,072.00	0.00	74,752.00	
15	UNIABDN	31.50	144,900.00	0.00	22,400.00	100,380.00	267,680.00	0.00	210,000.00	
16	LARPRO	23.50	116,325.00	0.00	45,100.00	96,855.00	258,280.00	0.00	230,540.00	
17	ARVI	38.00	171,000.00	40,000.00	36,000.00	124,200.00	371,200.00	0.00	284,400.00	
18	CHG3	26.00	65,000.00	0.00	180,000.00	147,000.00	392,000.00	0.00	298,400.00	
19	TNET	18.00	97,507.00	0.00	54,000.00	90,904.20	242,411.20	0.00	188,542.00	
20	HERMES	19.10	85,950.00	0.00	27,500.00	22,690.00	136,140.00	0.00	105,390.00	
21	NEDERLOF'S	19.10	85,950.00	0.00 - Workplan table	21,600.00		129,060.00	0.00	100,080.00	

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### WT8: Project Effort and costs

			Estimated						
Benefi- ciary number	Beneficiary short name	Effort (PM)	Personnel costs (€)	Subcontracting (€)	Other Direct costs (€)	Indirect costs OR lump sum, flat-rate or scale-of-unit (€)	Total costs	Total receipts (€)	Requested EU contribution (€)
	Total	591.60	1,913,712.00	89,000.00	1,099,400.00	1,954,629.20	5,056,741.20	0.00	3,986,295.00

#### 1. Project number

The project number has been assigned by the Commission as the unique identifier for your project. It cannot be changed. The project number **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

### 2. Project acronym

Use the project acronym as given in the submitted proposal. It cannot be changed unless agreed so during the negotiations. The same acronym **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

#### 53. Work Package number

Work package number: WP1, WP2, WP3, ..., WPn

#### 54. Type of activity

For all FP7 projects each work package must relate to one (and only one) of the following possible types of activity (only if applicable for the chosen funding scheme – must correspond to the GPF Form Ax.v):

• **RTD/INNO =** Research and technological development including scientific coordination - applicable for Collaborative Projects and Networks of Excellence

- DEM = Demonstration applicable for collaborative projects and Research for the Benefit of Specific Groups
- **MGT** = Management of the consortium applicable for all funding schemes
- OTHER = Other specific activities, applicable for all funding schemes
- COORD = Coordination activities applicable only for CAs
- SUPP = Support activities applicable only for SAs

### 55. Lead beneficiary number

Number of the beneficiary leading the work in this work package.

#### 56. Person-months per work package

The total number of person-months allocated to each work package.

#### 57. Start month

Relative start date for the work in the specific work packages, month 1 marking the start date of the project, and all other start dates being relative to this start date.

### 58. End month

Relative end date, month 1 marking the start date of the project, and all end dates being relative to this start date.

### 59. Milestone number

Milestone number:MS1, MS2, ..., MSn

#### 60. Delivery date for Milestone

Month in which the milestone will be achieved. Month 1 marking the start date of the project, and all delivery dates being relative to this start date.

#### 61. Deliverable number

Deliverable numbers in order of delivery dates: D1 - Dn

#### 62. Nature

Please indicate the nature of the deliverable using one of the following codes

 $\mathbf{R}$  = Report,  $\mathbf{P}$  = Prototype,  $\mathbf{D}$  = Demonstrator,  $\mathbf{O}$  = Other

#### 63. Dissemination level

Please indicate the dissemination level using one of the following codes:

#### • PU = Public

- PP = Restricted to other programme participants (including the Commission Services)
- RE = Restricted to a group specified by the consortium (including the Commission Services)
- CO = Confidential, only for members of the consortium (including the Commission Services)

• Restreint UE = Classified with the classification level "Restreint UE" according to Commission Decision 2001/844 and amendments

• **Confidentiel UE =** Classified with the mention of the classification level "Confidentiel UE" according to Commission Decision 2001/844 and amendments

• Secret UE = Classified with the mention of the classification level "Secret UE" according to Commission Decision 2001/844 and amendments

#### 64. Delivery date for Deliverable

Month in which the deliverables will be available. Month 1 marking the start date of the project, and all delivery dates being relative to this start date

### 65. Review number

Review number: RV1, RV2, ..., RVn

#### 66. Tentative timing of reviews

Month after which the review will take place. Month 1 marking the start date of the project, and all delivery dates being relative to this start date.

#### 67. Person-months per Deliverable

The total number of person-month allocated to each deliverable.

### PART B

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B2.2.14 PARTNER 14 INSTITUT ZA OCEANOGRAFIJU I RIBARSTVO (INSTITUTE OF CEANOGRAPHYAND FISHERIES), IZOR, CROATIA
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ABBREVIATIONS	AND ACRONYMS
ANOVAs	Analysis of variance
BIOHAZ	Biological Hazard Panel (EFSA)
BRC	British Retail Consortium
CODEX	Codex Alimentarius Commission
CSIC	Consejo Superior de Investigaciones científicas
CSIC (ICTAN)	(Institute of Food Science and Technology)
CSIC (IIM-E)	(Institute of Marine Research-Ecobiomar group)
CSIC (IIM-QPM)	(Institute of Marine Research-Marine Product Chemistry group)
CSIC (MNCN) DDGE	(National Natural History Museum) Denaturating gradient gel electrophoresis
EAACI	European Academy of Allergy and Clinical Immunology
EAS	European Aquaculture Society
EATIP	European Aquaculture Technology and Innovation Platform
EC	European Community
EDC	Exploratory and Dissemination Comiittee
EFSA	European Food Safety Agency
EFTP	European Fisheries Technology Platform
ELISA	Enzyme-linkes immunosorbent assay
ETPGAH	European Technology Platform for Global Animal Health
EU EURLP	European Union
FAO	European Reference Lab for Parasites Food and Agriculture Organization
FEAP	Federation of European Aquaculture Producers
FOB	Free on board
GC-MS	Gas chromatography-mass spectrometry
IFS	International Food Standard
HACCP	Hazard Analysis and Critical Control Points
ICES	International Commission for the Exploration of the Sea
ICPC	International Cooperation Partner Countries
lgG/E	Immunoblobulin G/E Interleukin
IL IFN	Interferon
IPR	Intellectual Property Rights
ISO	International Organization for Standardization 22000
ICT	Information and Communication Technologies
LED	Light-Emitting Diode
MSC	Marine Stewardship Council Certification
M	Million
Mt	Metric tones
mtDNA NAFO	Mitochondrial DNA Northwest Atlantic Fisheries Organization
NRLS	National Reference Lab for Parasites
ODBC	Open Database Connectivity
OIE	World Organization for Animal Health
PA	Project Assembly
PC	Project Coordinator
PCR	Polymerase Chain Reaction
PM	Personal/month
PT	Proeficient Test
QRA RASFF	quantitative risk assessment Rapid Alert System for Food and Feed
RD	Real Decreto
RE	Restricted to a group specified by the consortium
REA	Research Executive Agency
RTD	Research and technological development
SC	Steering committee
SESAM	European Commission online reporting tool for Research and Technological projects
SMEs	Small and Medium Sized Enterprises
SoA	State of Art
Th1/2 TNF	lymphocytes inducing cellular (1) and humoural (2) responses Tumor necrosis factor
USA	United States of America
UV	Ultraviolet
WEFTA	West European Fish Technologists Association
WHO	World Health Organization
WP	Western Blotting
WP	Work Package
WTO	World Trade Organization

### B1. Concept and objectives, progress beyond state of the art, S/T methodology and work plan

### **B1.1 Concept and Objectives**

The EU fishing industry is the fourth largest in the world. It provides some 6.4 million tonnes of fish each year. Fishing and fish processing provide jobs for more than 350,000 people. Moreover, the European fish market is one of the leading fish markets in the world with imports amounting to  $\leq$ 15 billions in 2009, more than 40% of world fish imports in value, and on an increasing trend. According to the FAO the average consumption of fish in the EU-27 countries is 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 (Chai et al. 2005).

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 proposal will focus 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).

We have placed less emphasis on trematodes and cestodes in the present project because, although zoonotic flatworms infect freshwater fish in many parts of the world, consumption of freshwater fish in the EU is relatively low and localised. The European Reference Lab for Parasites has reported that, in the EU, the only parasites transmitted to humans through consumption of freshwater fish are the trematode *Opisthorchis felineus* (responsible for around 180 infections due to consumption of marinated tench fillets, mainly in Italy, since 2003) and the cestode *Diphyllobotrium latum* (the etiological agent of about 80-90 infections per year in the EU due to the consumption of raw or undercooked fillets of several perch and salmon species, mainly in Estonia, Finland, France, Italy, Lithuania, Poland and Romania).

It is well-accepted that the most effective treatment for seafood at risk from anisakid parasites remains prevention of the parasite reaching the human host. Regarding anisakiasis, prevention of sensitization is most likely to be effective by control of infection (i.e. reducing the likelihood of parasites being present in fish flesh ready to eat by humans). Thus, identification and treatment of fish, and catching fish from areas where the parasites are either absent or present at very low incidences are critical control points in the Hazard Analysis and Critical Control Point food-safety system (HACCP). Prevention of zoonosis in the food chain, from the net to the plate, requires active policies fueled by research and innovation in food safety and quality addressed towards consumer protection within a high-standard EU market. This is important not only for

dietary recommendations, but also for its implications in preventive public health interventions according to the hazard identification and the exposure assessment to that given hazard.

In the PARASITE project, aspects including knowledge of distribution and abundance of parasites and their antigens in fish (pre-and post-mortem) through fishery products, detection methodologies, diagnosis of *Anisakis* allergy, treatments for killing parasites (and inactivating their antigens) and risk assessment will be made available to help policy-makers and the industry to meet International, EU and National standards for food safety. The PARASITE project will address the issue of parasites in fish in line with EU legal scenarios defined by the zoosanitary regulations and the Hygiene Package (852-853-854/2004). The project will also develop and evaluate technology and methods use by the industry in relation to the regulatory framework mentioned above, but also to other international standards for seafood quality, safety and sustainability (i.e., ISO 2000, IFS, BRC, MSC, etc).

One of the major requirements of an industrially feasible and traceable prevention program is applicability to real-life operations from net to plate. By characterizing the risk of infection in exploited seafood stocks (i.e., with previous strong epidemiological knowledge on stocks) it would be possible to notably reduce the number of heavily infected fish being transferred to fresh, processing and post-processing markets, and consequently reduce health risks to fish workers and consumers, and time and costs for the industry. This would be achieved not only by increasing control strategies, but also by implementing new methods and tools for parasite detection, diagnosis, monitoring and mitigation throughout the production and market chain. The development of pre- and co-standard-settings would contribute to increasing the safety guarantees on international trade and consumer confidence on fish and seafood products and new opportunities for a rigorous labeling will come up (see EFSA opinion of 2004\* of the Scientific Panel on Dietetic Products, Nutrition and Allergies relating to the evaluation of allergenic foods for labeling purposes). It is known that some Anisakis allergens are highly resistant to traditional physical treatments (e.g. heat or freezing) and that some sensitized patients develop symptoms after ingestion of dead anisakid larvae (or even without such ingestion). Furthermore, it must be noted that some companies are now offering ("Anisakis-free") labelling on their fish products. This indicates that consumers are willing to pay for added-value information indicating that a fishing ground does not present a real health hazard with regard to the presence of parasites or, alternatively, for those treatments that ensure that parasite and their allergens have been removed. This is especially relevant to major European markets where a significant number of allergic reactions caused by zoonotic anisakids have been reported.

Knowledge on interventions in the food chain to reduce risk (focusing on technological treatments with industrial upgrading) and on immune responses to antigens (including sensitization to exposure), and quantitative risk analysis, are desirable inputs for rational management of parasitized fish stocks and products, both locally produced or imported into EU markets. This proposal will provide scientific evidence and technological developments that will address risks associated with fish-borne parasites and will therefore strengthen the competitiveness of SMEs operating in the fishery sector, contribute to food safety policy and to help increase consumer protection.

### B1.1.1 S/T Objectives

Within this framework, the general objective of the project is – 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 will be pursued by developing, refining and adjusting tools for the detection, monitoring and mitigation of the risk faced by consumers from zoonotic parasites in fishery

products in Europe. Following *the Guide of Food Safety Risk Analysis from FAO (2006)* we define detailed objectives which include the following "fit-for purpose" specific actions:

- Providing comprehensive epidemiological data to assess the risk for consumers to encounter zoonotic parasites present in fish stocks and fishery products in the EU;
- Implementing multilevel methodologies, techniques and technology with the potential for upgrading to industrial scale, to detect zoonotic parasites and their proteins in order to mitigate the risks from this hazard in EU marine fish production value chains;
- Improvements in the capabilities of the stake-holders (e.g., food business operators, policy makers, clinicians etc) to use the epidemiological data, diagnostic tools, methodologies and devices resulting from the project, supported by a comprehensive dissemination strategy.
- Quantitative Risk Assessment (QRA) will be performed in conjunction with statistical modelling and Monte Carlo simulations to estimate the probability of illness from parasites associated with the consumption of raw or partially cooked fish products. The QRA will quantify the associated uncertainty and variability and estimate the effectiveness of proposed risk mitigation strategies. Cost-benefit analysis of proposed solutions will be also undertaken.

### B1.2 Progress beyond the State of the Art

### The EU fish market and the imported zoonotic parasites

Fisheries and aquaculture are important food production systems in Europe. In recent years, the EU fish industry has been increasingly dependent on imports of fish and fishery products to meet its needs. In 2009, the EU imported €15.5 billion worth of fish and fishery products, accounting for more than 60% of its fish consumption (http://ec.europa.eu/trade/creating-opportunities/economic-sectors/fisheries/statistics/).

Several major European markets provide good examples of new or re-emergent imported fish products in various commercial displays. Tuna fish is known to be a host of anisakid nematodes. In spite of the zoonotic implications of these parasites for worldwide trading of marine fish, no protocols have been carried out to assure the absence of anisakid infections in these marine fish. The difficulty of inspection of infected fish, especially for nematodes that have migrated into the body muscles of the fish, makes it difficult to assure the safety and quality of fishery products and creates risks for human consumption. Moreover, the occurrence of parasites of zoonotic or aesthetic quality importance such as Anisakis spp. may have a significant negative impact on consumer perception of these valuable resources. Due to the large size of many tuna species, larvae of Anisakis spp. are thought not to be able to cross the stomach wall and thus rarely reach the visceral organs or the flesh of the fish (Mattiucci pers. com.). Therefore the risk of human infection by Anisakis from tuna has been considered relatively low. However, there is still a lack of scientific evidence to confirm this assumption. The PARASITE project includes a task (WP2) to investigate the occurrence and spatial distribution of Anisakis spp. in tuna species from the Mediterranean Sea and NE Atlantic waters, and in Philippine tuna bound for export. Special emphasis will be placed on the fillets since tuna is regularly consumed raw or in a slightly smoked state, thus increasing the consumer risk from the possible presence of *Anisakis* larvae in tuna.

One of the products increasingly imported to Europe during the past decade is frozen fillets of cultured *Pangasius* catfish, the most important being the Sutchi catfish (*Pangasianosus hypophthalmus*). Recent studies have shown that intensively pond-cultured Sutchi catfish from the Mekong Delta area are regularly infected with at least four species of potentially zoonotic flukes

(Trematoda, Digenea) (Thuy et al. 2010). For example, the potentially human infective developmental stage (metacercariae) of the species *Haplorchis pumilio*is present in the flesh of  $\geq$  5 % of pond-cultured catfish. This implies that consumption of raw or lightly processed flesh of these fish, especially parts of the fillets close to the fins, constitutes a comparatively high risk of acquiring *H. pumilio* infection (Skov et al. 2009). Although Vietnamese *Pangasius* are usually exported as frozen fillets, this situation may change due to steadily increasing cost- and time-efficient air transport to a range of overseas export destinations including Europe. This again emphasises the need to conduct comprehensive epidemiological investigations on cultured *Pangasius* and its products, covering all seasons and representative production systems. The PARASITE proposal will address this objective in WP2.

Another major imported seafood product to Europe is cephalopods. The increasing depletion of many major fish stocks that formerly supported industrial scale fisheries forces continued attention to the once-called "unconventional marine resources" such as cephalopod species. Cephalopod catches increased steadily from 1 million in 1970 to more than 4 million tonnes worldwide in 2008, the Japanese short-finned squid Todarodes pacificus representing more than 10% (mainly 400,000-750,000t annually from 1950). Considering that European consumers have increasingly adopted the Asiatic dietary custom of eating raw fish and seafood such as shrimps, bivalves, gastropods and cephalopods, they are at risk of infection with human pathogenic parasites present in these aquatic organisms. In Japan, numerous cases of infections by parasites of T. pacificus have been reported (Nagasawa & Moravec, 1995). Four species of helminth parasites have been reported to be infective to humans, the nematodes Anisakis simplex, A. physeteris, Pseudoterranova decipiens, and the cestode Nybelinia surmenicola. Of these, A. simplex is the most relevant from the epidemiological perspective since more than 12,000 cases of human gastric and intestinal anisakiosis were reported up to 1989 in Japan (Ishikura et al. 1993). The increasing importance of T. pacificus in European markets and the RASSF (Rapid Alert System for Food and Feed) notifications related to cephalopods underline the importance of undertaking a case study targeting *T. pacificus* within the context of our proposal (WP2).

### Epidemiology of zoonotic nematodes

Regarding domestic fish resources in European markets, public awareness of the possible presence of parasites in fish products is reflected by the increasing number of notifications of parasites in fish under the ECs RASFF (http://ec.europa.eu/food/ food/rapidalert/index\_en.htm) (Figure 1). The parasites of primary concern are the larvae of several species of parasitic nematodes (roundworms) (e.g., *Anisakis* spp., *Pseudoterranova* spp., *Contracaecum* spp.), with *Anisakis simplex*, commonly known as the herring- or whale worm being the most prominent.

Anisakid larvae are common in virtually all marine fish species inhabiting EU fishing grounds. While the majority of larvae – regardless of fish host species – seem to lodge on the organs and body wall of the abdominal cavity, some worms migrates into the flesh of the fish, sometimes deeply into the fillets (Figure 2). This behaviour, which makes detection and removal of worms more difficult, is to blame for the attention the worms receive from consumers and public food safety authorities. In this respect, the species *Anisakis simplex* s.l. appears to be the parasite of greatest concern.



Figure 1. RASFF Anisakis notifications over the time period 1/2006 - 9/2009.



Figure 2. Heavy anisakid infections in the viscera (left) and muscle (right) of a fish.

In fish and squid, the majority of anisakid larvae are typically encapsulated as flat and tight coils, measuring a few mm across. Larvae residing in fish flesh are very hard to detect using visual inspection because of their often transparent/whitish appearance. Many native or imported fish species marketed in Europe carry anisakids with zoonotic potential, sometimes with high prevalence in the edible part of the fish. Much of the available information on these parasites originates from nationally funded epidemiological studies of anisakids in regional fish stocks and products. Anisakid species commonly occur in fish at least two ecologically different environments, i.e. pelagic/oceanic and benthic/coastalecosystems. The habitats of these two ecosystems are characterized by different physical conditions and different key species with respect to zooplankton, fish and marine mammals. Among the commercially most important pelagic fish species are herring (Clupea harengus), Atlantic mackerel (Scomber scombrus), blue whiting (Micromesistius poutassou) and anchovy (Engraulis encrasicolus) while European hake (Merluccius merluccius), haddock (Melanogrammus aeglefinus) and Atlantic cod (Gadus morhua) are currently the economically most important benthic/demersal species in European fishery industries. Additionally, Monkfish (Lophius sp.), Scabbard fish (Aphanopus carbo) and Sea bass (Dicentrarchus labrax) are commercially utilised on an industrial scale and are of importance in a number of major European seafood markets including Spain, Italy and France. It is scientifically well documented that all of these fish species or stocks are infected with different species of anisakid nematodes. However, the lack of a Pan-European systematic monitoring and established sampling programs for anisakids means that there is only little information on temporal and spatial trends in the population dynamics of these parasites in economically important fisheries in European waters. EFSA therefore recommends that surveillance of fishery products for infection by anisakids is increased (EFSA Journal 2010; 8(4):1543).

To date, nine species of the genus *Anisakis* have been genetically detected and described (Mattiucci & Nascetti, 2006, 2008; Mattiucci et al., 2009). Two of them, i.e. *A. simplex* s.s. and *A. pegreffii* have been so far recorded by molecular methods as causing human anisakiasis

(Umehara et al., 2007, Mattiucci et al., 2009, 2011a). *A. simplex* s.s. and *A. pegreffii* are the main zoonotic parasite species occurring in several fish and squids of European water (for reviews, see Mattiucci & Nascetti, 2006; 2008), often also co-infecting the same fish host in sympatric areas. However, other *Anisakis* species have been recorded in European fish and squids by several authors, i.e. *A. physeteris, A. typica, A. ziphidarum, A. brevispiculata, A. nascettii* (Mattiucci & Nascetti, 2008). It has been found that different species of *Anisakis* have a differential geographical distributions, host preference, and possibly different life-cycles. To date, *A. simplex* C, *A. ziphidarum, A, nascettii* and *A. brevispiculata* have been recorded in fish and squid from Pacific waters. However, the risks to consumers posed by seafood products from those fishing grounds which reach European markets, and the potential zonootic role of some of those *Anisakis* spp., have never been investigated. One of the aims of this project will focus on this aspect, providing molecular epidemiological data concerning fish and squid species collected from those areas (WP 2 linked to WP4).

The factors and mechanisms that govern the occurrence and distribution of anisakid nematodes in the NE Atlantic and adjacent seas including the Mediterranean and Baltic Seas are still largely unknown. Self-control programmes in the fish industry are hampered by the fact that the epidemiology of fish nematode parasites in European markets is not well-understood. The collection of systematic data on the complete life cycle, geographical and seasonal distribution, prevalence, intensity, and anatomical location of parasites of public health importance in wild caught stocks and fishery products is currently made on biased and opportunistic bases, with no time-series available nor any monitoring programs coordinated on a pan-European scale. A welldesigned systematic epidemiological survey (WP2) of the ten economically most important fish species or stocks from four major fishing areas aims to provide the basis for analysing and modelling parasite prevalence. Besides the large scale of the survey in terms of geographical and temporal range as well as sample size and number of host species, an important advantage will be the use of the same nematode detection method by all surveyors to map the infection level and spatial distribution of anisakid larvae in the target fish species and fishing areas. This will reduce the effect of errors due to differences in detection efficiency and operator skills on the quality of data during sampling and registration. The Biobank solution (WP 3) will operate as a promoter of technical quality. Partners will be able to obtain parasite material with high standards for sample traceability and integrity. It will ensure availability of high guality biological material, well sorted, processed and preserved to meet the demands of research, and to facilitate the future development of diagnostic systems that enable precise classification, forecasting methods and identification of potential therapeutic targets.

Recent findings indicate that *A. pegreffii* can reach the muscle of living fish (Suzuki et al., 2010). This aspect could acquire importance in defining the risk assessment related to different fish hosts, fishing grounds, etc. One of the aims of the project is to provide a web-based platform to present the relevant epidemiological information in a user-friendly format for the end-users (WP3). This approach will constitute the first baseline risk analysis for inspectors. Additionally, this tool may help food producers to maintain safety, consistency and avoid consumer complaints or costly litigation because of rapid alert notifications.

### Public health concern

Humans can indeed accidentally be infected by the anisakid larvae when consuming raw, undercooked or improperly processed (e.g. marinated) parasitized fish and squid. In humans, these parasites do not mature, but they provoke the zoonosis termed anisakiasis. Several nematode species are implicated in this disease. Among the anisakid nematodes, larvae of the genus *Anisakis* are more important than those of the genera *Psuedoterranova* and *Contracaecum*
in provoking the human disease. Indeed, most human cases of the zoonosis were found to be provoked by *Anisakis* spp. larvae. However, several cases have been also reported as due to *Pseudoterranova* larvae (Mattiucci, per. comm.). Some cases are found to be related also to *Contracaecum* larvae.

In Europe, human infection with live anisakids most frequently reported from certain regions of Spain and Italy where raw, lightly salted or marinated fish is part of the regular diet. In human infections, these parasites can migrate from the gastrointestinal tract, becoming embedded in the gastrointestinal mucosa and submucosa. This presents clinically as sudden onset of severe, episodic, epigastric distress, sometimes accompanied by nausea and vomiting. Acute epigastric pain generally occurs within 1-12 h of consumption of the infected seafood and eosinophilia has been reported in the chronic stage of the disease. In some cases the larvae may be coughed up in an otherwise symptom-free person. Based on the location of the granulomatous lesions, various types of human anisakiasis have been identified, most being either gastric or intestinal. Here, larvae penetrate the tissues and cause severe pathology. Extra-gastrointestinal cases (e.g. oropharyngeal) cases of human anisakiasis have been also reported. From the histopathological point of view, anisakiasis has four stages: i) phlegmon formation; ii) abscess formation; iii) abscess-granuloma formation; and iv) granuloma formation.

The other common manifestation of human anisakiasis is an allergic reaction in sensitized individuals. Gastric allergic anisakiasis (GGA) can arise when anisakid larva occur alive in the stomach, provoking allergic reactions of various degree and nature but even the ingestion of dead worms in food fish may cause severe hypersensitivity reactions. Such patients are typically advised to avoid seafood after diagnosis. Anisakiasis has also been reported to cause intestinal pseudo-obstruction, occupational asthma during filleting or cooking, peritonitis and rheumatologic symptoms (arthralgias/arthritis) (Audicana & Kennedy, 2008).

Although clinical aspects of the human disease are well-known, the role played by different species of anisakid nematodes in pathogenesis is not clear. In other words, different species of *Anisakis* and anisakids could be related to different aspects of the pathology and immunopathology of human anisakiasis. This topic requires further investigation: one of the aims of the project is indeed to investigate, throughout the experimental infections, the role played by different species of anisakids (of the genera *Anisakis, Pseudoterranova* and *Contracaecum*), when genetically characterized and identified (WP4). Indeed, an integrated approach to study these aspects, including the characterization of species-specific antigens (WP5) and their role in the experimental infection has never been carried out.

The taxonomy of zoonotic anisakids of the genera *Anisakis, Pseudoterranova* and *Contracaecum* has been recently reviewed (Mattiucci & Nascetti, 2008; Mattiucci *et al.*, 2008, 2009). Previously, systematics has leaned heavily on morphology but this has been readdressed in the last 25 years by the use of genetic-molecular methodologies. The application of molecular/genetic markers has allowed both advances in systematics (e.g. detection of sibling species) and, of particular importance in the context of the present project, the identification of larval stages of these nematodes to species level (Mattiucci & Nascetti, 2008).

In the molecular systematics, the application of genetic markers obtained from allozymes (the first genetic method applied) has allowed the discovery and genetic characterization of all the species belonging to those genera so far recognized, and the exact identification of their larval stages. Subsequently, those species have been also genetically characterized based on other genes (both nuclear and mitochondrial) allowing development of further molecular/genetic markers based on PCR-DNA to be used for the recognition to the species level, including the mtDNA *cox2* gene (Valentini et al, 2006; Mattiucci et al., 2008, 2009, 2011a,b), ITS-rDNA (D'Amelio et al., 2000) and ssrDNA (Nadler et al., 2005). In the present project, the above mentioned markers will be used for genetic identification of the zoonotic parasites and to gather information about population

structure of anisakids. Therefore, this approach represents the base to gather epidemiological data concerning the occurrence of potential zoonotic parasites. Some anisakid species infecting fish/squids may not be pathogenic to humans and their recognition acquires particular importance. Moreover, the application of genetic tools facilitates a wider epidemiological survey at large scale with an integrated approach. Indeed, all project activities related to the detection, antigen/allergen characterization, mitigation, etc., will benefit from the use of material (parasites) identified genetically to their species level. This represents an innovative aspect of the proposal (WP4) and will provide scientific data in support of new methodologies applicable in industrial or clinical contexts. Moreover, in the case of *A. pegreffii* and *A. simplex* s.s., the main species responsible for the zoonosis in humans and probably the most widespread in wild fish in European waters, new molecular markers, obtained from DNA microsatellite loci, will be developed and applied. This is innovative because such markers have never been applied on these parasites.

Finally, data on genetic variability gathered from the application of genetic markers, will be correlated to their distribution and abundance in different fish host species and geographical areas (WP4 linked to WP8). This represents another innovative aspect of the research. Indeed studies linking infection dynamics to the genetic variability of these parasites as a further tool to monitor their occurrence and population size in a given geographical area, have never been carried out. This will also provide a further tool to assess risks related to a fish species and fishing area.

Other anisakid larvae causing a negative impact on fisheries of the NE European countries are those belonging to the genus *Pseudoterranova*, which are often found in the flesh of several species of commercial importance. By means of genetic methodologies, it has been demonstrated that *Pseudoterranova decipiens (sensu lato)* from the North Atlantic Ocean and Norwegian and Barents seas comprises three sibling species, namely *P. decipiens s.s., P. krabbei* and *P. bulbosa*. These species have different host preference, with different species of seals as definitive hosts, and different geographical distributions. They are genetically distinct from *P. azarasi* from the North Pacific waters (Mattiucci & Nascetti, 2008).

The present project addresses risk assessment related to the occurrence of species of *P. decipiens* s.l. complex in fish fillets in both European waters and from the Pacific Ocean. Moreover, the possibility to study the zoonotic potential of different species of this complex to humans, through experimental infection of animal models (WP5) is also innovative.

*Contracaecum osculatum* (s.l.) is another anisakid of possible public health importance (Mattiucci et al., 2011b). It has been demonstrated to comprise at least five sibling species (reviewed in Mattiucci & Nascetti, 2008). To date, the siblings identified as occurring in fish from European waters are *C. osculatum* A and *C. osculatum* B, in the North Atlantic. In the Baltic Sea, *C. osculatum* s.s. is the only species present. Fagerholm (1988) showed that, in rats infected with third-stage *Contracaecum* larvae from fish cultured in vitro, fourth-stage larvae developed in the stomach at 2-5 days post-infection but no adult worms developed. Larvae introduced surgically into the body cavity of laboratory rats were later found deeply embedded in the gastric submucosa and in the peritoneal cavity, and yielded some adult worms from day 42 onwards. Infected rats with *Contracaecum osculatum* larvae collected from whiting and recovered larvae from 4 hours to 10 days post-infection. After 4 hours post-infection the majority of larvae were firmly embedded in the stomach wall, associated with a strong inflammatory reaction with necrosis and ulceration. Sporadic human cases of human anisakiasis have been reported due to the larval stages of *C.osculatum*, including at least one recent example (Shamsi & Butcher, 2011).

#### Technical limitations of existing products and systems for parasite detection.

In response to the burden of human disease experienced over the past 2–3 decades in several European countries (e.g. Germany, Romania, Spain, Italy), the 'Hygiene Package' (EC regulation 853/2004) and the member state regulations (e.g., RD 1420/2006) have included a range of preventive control measures to be applied by the industry and social food services in order to minimize the risk to human health from the possible presence of parasites in fishery products. This was generally implemented through *Hazard Analysis Critical Control Point* (HACCP) programs in the production and processing of fish. This standard-setting methodology supports normative food safety management systems (ISO 22000) for any organization involved in the food chain.

With a "post-harvest focus" the limiting factor is the absence of a recognised standard to ensure practical application of research results to the market. At the present, the industry's coresponsibility implies that the products have been subjected to a visual inspection to detect visible parasites before market release. Fishery products that are obviously contaminated with parasites must not be placed on the market for human consumption (EC 853/2004, Section VIII, Chapter V, Pt. D). However, due to economic and technological constraints, it is currently impracticable to detect and subsequently remove all parasites that might be present in the fillets of wild-catch and industrially processed fish. This is further underlined by the fact that no technique exists that is efficient and accurate enough to be implemented and accepted by the industry as a routine technique for product inspection regarding parasites.

Non-invasive methods like visual inspection and candling have been widely used in the industry for parasite screening in seafood over the last 50 years. In fact, they are regulated (EC/853, 2004; EC/854, 2004; EEC/140, 1993). These methods have been shown to be relatively ineffective and also have the disadvantage that they cannot be applied for the analysis of processed products (Levsen et al., 2005). New invasive methods like artificial digestion of fish samples for whole parasites (e.g., Tejada et al, 2007), PCR-based techniques for detecting trace amounts of parasites (e.g., Espiñeira et al., 2010; Mossali et al., 2010; Lopez & Pardo, 2010; Herrero et al., 2011) or immunological detection of parasite antigens (e.g., Rodriguez-Mahillo et al., 2010) are much more robust, highly specific and sensitive (e.g., 40 ppm, parasite in 25g of fish sample). These methods are readily applied to all kinds of processed seafood products, and are easily adaptable for the testing of batches of fish products in laboratory surveys. These techniques are much more appropriate for inspection bodies and lab-users. However, bearing in mind the substantial number of enterprises operating in European fish markets and the enormous amount of seafood that is landed and sold, these methods are inappropriate for on-site self-control inspections in SMEs because they are time-consuming and/or costly in terms of equipment, consumables and specialized personnel.

The UV-Press method is increasingly applied during systematic detection of nematode larvae in the flesh of fish, especially in large-scale scientific surveys (Levsen & Lunestad 2010). The method utilises the fluorescence of frozen anisakid larvae and is based on visual inspection of flattened/pressed and subsequently deep-frozen fish fillets or viscera under UV-light (Karl & Leinemann 1993). Prior to the pressing process, each fish is gutted and manually filleted before placing the visceral organs and both left and right-side flesh (fillets incl. belly flaps) into clear plastic bags. The samples are then pressed to 1–2 mm thick layers in a hydraulic or pneumatic pressing device (holding time 5 sec at 8-14 Bar). The bags containing the pressed fillets or viscera are then deep-frozen prior to visual inspection under a 366 nm UV-light source. The UV-light set-up should be equipped with both up- and down-light. Any anisakid larvae present appears as fluorescent spots in the samples, the brightness probably depending on various factors such as anisakid species involved, their size and age, and the extent of encapsulation (WP6). Besides the generally high detection score, the method also facilitates determination of the approximate infection site of the larvae, i.e. whether they are situated in the fillets or ventral portion (belly flaps) of the fish flesh

(Levsen & Lunestad 2010). The method may also be used for parasite inspection of larger fish such as cod or monkfish. In these cases, each fillet or fish side has to be cut into smaller sections which are then processed separately.

For routine inspection of fish or fishery products for nematode parasites, the pressing technique should be the method of choice. It is characterised by a high detection score and allows the examination of a comparatively large amount of samples in a comparatively short period of time. To meet the requirements of the industry for an efficient and user-friendlyprocess for routine parasite inspection of fishery products, various refinements need to be implemented. These will include automation of the pressing and parasite detection steps, and studies to optimise the thermal treatment necessary to activate the fluorescence-generating enzymes. This will be an important task in WP6.

Although it is generally agreed that the long-term goal is to develop a non-destructive method for fish during parasite inspection, a realistic approach for the seafood industry is to develop a broad range of online and lab-based applications. Thus, the epidemiological screening of fish stocks and products (WPs 2 and 3) represents a good basis for risk analysis while the UVpress method (WP6) is more suitable for industrial scale screening. These two technical solutions should be designed with high-reference applicability, even if they are not accredited by an international standard organization. The refinement of existing methods, their validation by interlaboratory trials and the beta-testing in SMEs (WP6) are necessary to guarantee the achievement of the co-responsibility criteria adopted in the EU Hygiene Pack. According to the ISO/IEC 17043/2010, the inter-laboratory comparisons should evaluate the performance of laboratories for the identification of problems and initiation of actions for improvement which, for example, may be related to inadequate test, effectiveness of staff training and supervision, or equipment. Moreover, the inter-laboratory comparisons (often described as collaborative trials) should also evaluate the performance characteristics of the method. As far as we know, only one proficiency test (PT) to detect Anisakis spp. larvae in fish fillets was organized in the European Union in 2009 (http://www.iss.it/crlp/docu/). The PT was organized by the EURLP to test the competence of the appointed National Reference Laboratories (NRLs) to detect Anisakidae larvae in fish fillets. Out of the 27 MS of the EU, 19 countries participated at the PT. Most of the participating laboratories (70%) detected all larvae presented in the samples.

Several approaches for the detection of *Anisakis* spp have been recently developed which have the potential of being certified by accredited European labs. These are based on the detection or quantification of parasite-derived material and utilise the detection of specific nucleotide sequences (Mossali et al. 2010, Lopez and Pardo, 2010) or parasite antigens and allergens (Rodriguez-Mahillo et al, 2010) in fishery products. These methods will also be optimized and/or harmonized in the proposed project in order to become standardized procedures complementing visual examination for parasite detection, and will be incorporated in accredited National or EU laboratories. This work will be undertaken in WPs 5 and 6.

A DNA - primer/probe system based on a real-time polymerase chain reaction (PCR) has been previously suggested as a detection assay for *Anisakis simplex* in seafood products (Lopez & Pardo, 2010, Herrero et al., 2010). The previously developed methodologies were based on the Taqman probe real-time assay targeting the mtDNA *cox1*, mtDNA *cox2* and ITS genes. However, the DNA-probes so far proposed were not designed for this purpose, are not species-specific for all anisakids and do not allow the identification of all zoonotic parasites to species level. Sequence analysis of several genes studied so far in anisakid species of the genera *Anisakis*, *Pseudoterranova* and *Contracaecum*, already completed by participants of the project, will allow setting up a species specific DNA probe for several of the species complex of the genera *Anisakis*, *Pseudoterranova* and *Contracaecum* (WP 6). The implementation and refining of the methodology based on selected genes will represent an upgrading step in the possibility to detect the presence of the parasite in seafood products.

#### Interventions in the food chain to reduce risk

Adequate management of seafood (i.e., post-processing control measures including cooking at 60°C for 10 min or freezing at -20°C for 24 h in the thermal center) is considered the safest way of preventing parasite-related infections. Nevertheless, there is no accurate information about other parameters which can affect the viability of the parasites (e.g. the heating or freezing method, the larvae (species and habitat) or host fish (fat, water, shape, etc) (WP 7). Some exceptions are cited to the heating conditions necessary to kill nematode larvae (Vidacek et al., 2010). Detailed information on the safe purchase, handling, and consumption of seafood may be obtained from national governmental and EU internet sites (e-g-http://www.aesan.es; http://www.efsa.eu.;http://europa.eu/legislation\_summaries/ other/l32041\_es.htm).

In recent years several techniques had been applied to fishery products to mitigate the risk of consuming infected fish products, by applying heat (conventional and by microwaves) (Vidacek et al, 2010), high pressure (Vidacek et al, 2009), acid and salt treatments (Solas et al, 2009), irradiation (Padovani et al, 2005), electrocution (Bererciartua, 2005), etc. Nevertheless, many of these treatments have not been tested in standardized conditions. Progress beyond the SoA for technological treatments to inactivate parasites and antigens is needed to ascertain how the different Anisakis species respond to the treatments and the role of the fishing area and habitat, the host fish and the storage conditions. This is the core of the tasks proposed in WP7. Additionally, WP7 will deal with contra-epizootic parasite measures during gutting and discarding operations on-board fishing vessels. The idea is to reduce parasite infection levels in wild fish (or at least to avoid increasing them) eliminating viable parasites from by-products discarded at sea, e.g. by treated offal with electromagnetic radiation emitted by a new Tech-device. A prototype for implantation on fishing vessels was built by a consortium with the participation of CSIC-IIM (see http://lp.espacenet.com/publicationDetails/originalDocument?FT=D&date=20110204&DB=lp.espac enet.com&locale=es LP&CC=ES&NR=2332858B1&KC=B1) but results on parasite inactivation and infectivity revealed the need for improvement, justifying proposed re-design efforts (WP 7).

#### Risk assessment

Human exposure to fish parasites depends on the incidence of parasites in wild seafood and on the treatments applied to seafood (e.g. filleting, freezing, irradiation, etc) prior to marketing to the consumer. Previous work has demonstrated the feasibility of using statistical modelling techniques to characterize patterns and trends in *Anisakis* abundance, e.g. in relation to fish species and size (e.g. Petrie et al 2007). There is however a need for wider-scale analysis. In WP8 we propose to integrate data on a range of zoonotic parasites from several commercially important fish and shellfish species across Europe, to establish spatial, temporal (seasonal and interannual) patterns and relationships with fish population density and environmental conditions, while also quantifying the typical pattern of parasite incidence within populations of the main fish species in relation to sex, size, age and maturity. Rapid developments in statistical modelling in the last decade mean that it is now feasible to take into account non-normal distributions of response variables, non-linear relationships with multiple explanatory variables (and interactions between them), spatial/temporal autocorrelation in data series, zero-inflation and nested data sets within traditional or Bayesian statistical frameworks (Zuur et al., 2007, 2009). WP 8 will explore this approach as a tool to improve understanding and prediction of nematode abundance.

Quantitative risk assessment (QRA) is a technique which is used to estimate the likelihood and severity of an adverse event. When performed in conjunction with Monte Carlo simulation, it offers precise explanation of the uncertainty and variability associated with the risk (Vose, 2000). Quantitative microbial risk assessments have been used to estimate infection rates in humans through the food chain (e.g. E. coli O157 in beef burgers, Listeria monocytogenes in smoked salmon and trout), the water supply and the environment (e.g. Lindqvist & Westoo, 2000). The risk assessment requires an initial statement of purpose and the process involves four primary stages: (1) hazard identification, which identifies the pathogenic organism of concern and whether it is actually a hazard in the context that it is being studied; (2) exposure assessment, to determine the number of organisms ingested; (3) hazard characterization, which gives a quantitative or qualitative assessment of the adverse effects of the pathogen to humans; more specifically a dose-response model can be implemented which mathematically models the variability in impact (response) following exposure to different doses; (4) risk characterization, which gives a probability of occurrence of the illness and also the severity of the health effects in a given population. In WP8 we apply this methodology to determine the probability of illness from parasites associated with the consumption of raw or partially cooked fish products and estimate the effectiveness of proposed risk mitigation strategies.

Quantifying the costs and benefits of reducing parasite incidence in seafood has several facets. There is an obvious benefit in terms of human health, albeit probably largely restricted to areas where outbreaks of anisakiasis, etc, normally occur. It is also evident that the visible presence of parasites in seafood is sufficiently strong motivating factor to significantly reduce consumption of fish products at least in the short-term. There is a need to evaluate scenarios based on potential changes in policy and practice for parasite inspection and control methods applied to seafood. It is not known how much consumers are willing to pay for treatments which will remove parasites from fish. The main objective of the willingness to pay analysis is to identify how individuals value the various attributes of a product or service, such as fishery products. In the context of this proposal, respondents (consumers) will be presented with, and asked to make choices between, alternative hypothetical fishery products involving different levels or methods of treatment for parasite elimination that have been identified as important for influencing the quality of the fishery product (attributes). We assume that a treatment for parasite elimination may be adequately described by "i" attributes. Hence, a fishery product may be described by a vector, the so called 'product vignette'. Individuals will then be offered a list of vignettes, and asked to rank those in order of preference and/or to evaluate them on a numerical scale or in terms of verbal labels, varying from 'very bad' to 'very good'. In this manner, the respondents are forced to trade-off some characteristics for others and to incorporate opportunity cost into their decision-making process, akin to the way that they make decisions in the real world. The vignettes will be analysed in terms of how sensitive the answers are with respect to changes in the vignette descriptions. This methodology is widely used in relation to consumer references in a variety of contexts (e.g. Chern et al. 2002). It needs data collection that allows the researcher to disentangle individual preferences based on information that they state in a questionnaire. It is a stated preference methodology that is rooted in random utility theory.

## B1.3 S/T Methodology and associated work plan

## B1.3.1 Overall strategy of the Work plan

The main objective of the proposal is to provide further understanding of food safety and quality aspects regarding the presence of zoonotic parasites in fish stocks and products marketed in EU.

The management concepts and coordination utilities for that objective will be developed in WP1, strategically based on a Steering Committee.

The proposal focuses on three innovative cooperative challenges (SMEs competitiveness, food safety policy and international collaboration) transversally covered in WP 2 to 7 which will work closely together to identify and characterize the hazard and to mitigate the risk (Figure 3). Introduction on the market of safe and high-quality fish products requires harmonizing methods and developing assessment tools exploiting potential synergies and combinations which are needed for a continuous condition monitoring, information collection and exchange, for predictive and risk-based maintenance of self-control programs and policies. The integration of results (i.e, the synthesis) from WPs 2-7 will take place in WP8 and WP9 driven by the Dissemination Committee, the end-users and the external Advisory Panel.

The project is designed to incorporate risk assessment principles into the decision-making process for an effective food safety management of parasite hazards present in fishery products. To that end, the project will concentrate in elaborating a risk profile for seafood-borne parasite hazards of current concern establishing the WPs as the required components for the initial statement of a risk assessment as the ·science-based" component of risk analysis. The WP2 (parasite exposure assessment), WP4 (hazard identification) and WP5 (hazard characterization) will be the primary stages of the risk assessment. They will generate transversal results, i.e. improved data and tools (WP3) which will be used to undertake secondary stages (detection methods in WP6 and interventions in the food chain in WP7) for risk assessment, either qualitative and quantitative (WP8). The generated knowledge will reach the end-users by technology transfer, communication and dissemination capabilities that will be designed and implemented (WP9) as an added-value active of the proposal.

Within the WPs we will define several tasks to face the gaps on available knowledge identified by the EFSA's Biological Hazards (BIOHAZ) Panel on the Scientific Opinion on risk assessment of parasites in fishery products (EFSA, 2010). Many tasks will provide scientific evidence and technological enhancement on new data, methods and devices to a better understanding of the distribution of parasites in fish body, detection methodologies in fishery products, sensitization and exposure to A. simplex, diagnosis of Anisakis allergy, human genetics and the immune repertoire, development and activation-associated nematode allergens, antigen exposure through fishery products, epidemiological data, assessing viability, evaluation of treatments defined by legislation to kill viable endoparasites, evaluation of alternative physical treatments compared to the freezing method described in the hygiene Regulations, assessing health hazard related to the presence of parasites from fishery products of different origins and production methods, monitoring and surveillance systems in fishing grounds and geographical distribution. The achievement of deliverables and milestones within each WP will represent a roadmap to help health professionals, people working in the fish industry and the general public with information on the risks resulting from these parasites, as well as on best methods to eliminate, thus improving the seafood safety and quality aspects of fish European markets. This will strength the competitiveness of the fishing industry, it will help to define active prevention sea food policies, and it will guarantee a high-quality standard of consumer protection.

The Work plan designed to achieve the objectives has been split into a total of 9 Workpackages (see below).

Work Packages:	Objective:
WP 1: ADMINISTRATIVE PROJECT MANAGEMENT	To enable effective administrative project management by implementing the most appropriate tools and means that will guarantee a fluent exchange of information and an efficient and transparent decision-making process, including IPR management. This also aims to assure efficient project reporting and satisfactory monitoring, accomplishment and follow-up of all project items, especially milestones and deliverables.
WP2: EXPOSURE ASSESSMENT	To provide comprehensive and comparable epidemiological data on zoonotic parasites in fish stocks originating from major European fishing grounds; To map zoonotic parasites in key fish product imports of relevance to European markets.
WP3: SAMPLE & DATA MANAGEMENT	To provide management tools for traceability and high-quality storage of samples to be used in diagnosis, trials and experimental challenges within the project; To implement a scientific and technological-based Biobank for zoonotic parasites in fishery products; To implement a computer-aid epidemiological geo-referenced database for zoonotic parasites in fish stocks and products marketed in Europe, including development of assessment utilities for end-users.
WP4: HAZARD IDENTIFICATION	To use genetic markers to identify and characterise species and populations of zoonotic nematode parasites infecting fish and cephalopod species and products from different geographical areas; To develop new genetic markers for genotyping <i>Anisakis</i> species; To identify genes and design primers/probes for use as "DNA barcodes". To gather genetic variability data of parasites populations, to be correlated to their infestation levels in order to establish scientific bases for molecular epidemiological studies of each parasite species and their populations in different geographical areas.
WP 5: HAZARD CHARACTERIZATION	<ul> <li>To determine if other parasites of the Family Anisakidae (i.e. apart from <i>Anisakis</i> spp.), that can infect the muscle of fish after migration from the coelomatic cavity:</li> <li>have allergenic capacity</li> <li>are able to induce sensitization after oral administration, whether untreated or heat-treated</li> <li>are specifically recognized by antibodies presented in sera from fish eating people.</li> <li>To detect <i>Anisakis</i> spp allergens in fishery products. To detect allergens (or potential allergens) in samples of anisakids from different regions; To characterize cellular and humoural immune responses to anisakid antigens.</li> </ul>
WP6: IMPROVEMENT OF DETECTION METHODS FOR THE INDUSTRY AND OTHER END- USERS	To improve visual, ultraviolet and molecular inspection methods for detection of parasites of human health significance in fishery products, making them usable by industry, research bodies and sanitary authorities
WP7: INTERVENTIONS IN THE FOOD CHAIN TO REDUCE RISKS	To determine methods to assess viability and infectivity of anisakids in commercial products under different treatments and conditions; To obtain evidence on the interactions between parasites and bacteria in the flesh of post-harvest fish under different storage conditions; To design optimal treatments for the inactivation of anisakids in fishery products; To develop specific treatments to reduce or inactivate the allergenic capacity of

	F
	anisakids; To develop an on-board prototype method to kill
	zoonotic anisakids in fish offal.
WP8: QUANTITATIVE RISK ANALYSIS	Summarize, analyze and predict consumer exposure to fish parasites through statistical modelling of patterns and trends in the incidence of zoonotic parasites in commercially important fish species (and in seafood products); Develop a dynamic framework to integrate parasite infection and genetic variability estimates in order to predict parasite abundance; Collate and analyze available data on the incidence of parasite infections in humans to assemble a picture of the geographical and temporal patterns and identify hotspots; Undertake a quantitative risk assessment, with a particular focus on the probability of illness from parasites associated with consumption of raw or partially cooked seafood products and estimate the effectiveness of proposed risk mitigation strategies; Model consumer willingness to pay for treatments to reduce incidence of parasites in fish products; Define and evaluate Cost/Benefit scenarios on the application of treatments and tools for policymakers and food producers.
WP9: INNOVATION, COMMUNICATION AND DISSEMINATION	To achieve effectiveness in communicating the risks associated with fish/seafood parasites targeted in this project, increasing transparency, enhancing the benefits of fish consumption and explaining the measures and possible paths and tools to tackle such risks; To reinforce the competitiveness of the seafood industry by improving its skills and capacity to use the project results and implement strategies to tackle parasite-related risks; To spread the knowledge achieved and disseminate results to the different stakes holders: scientific bodies, policy-makers at European, national and regional level, to the industry, and to consumers and civil society.

																								F	PAF	RAS	ITE :	3120	68
B 1.3.2 Timing of work packages and their components	1	2	3 4	5	6	7	8 0	10	11	12 1	13 14	15	16 1	17 18	19	20	21	22 2	3 24	25	26	27	28 2	29 30	31	32	33	34 35	36
WP 1: ADMINISTRATIVE PROJECT MANAGEMENT		2				Ĺ		10		12	15 14	1.5	10 1		1.5	20	21		5 24	2.5	20							34 33	
Task 1.1 Consortium management	x	D1.1	x x	x	x	x	x >	x	x	x	x x	x	x .	x x	x	x	x	x >	( x	x	x	×	x	x x	×	x	x	x x	x
Task 1.2 IPR management	x	x	MS1 x	x	x	x	x >	x	x	x	x x	x	x	x x	x	x	x	x )	t X	x	x	×	x	x x	×	x	x	x x	x
WP2: EXPOSURE ASSESSMENT																													
Task 2.1 Surveillance of zoonotic parasites of commercial key fish species and products from European fishing grounds.	x	x	D2.1 x	x	x	x	x	x	x	x	x x	x	x	x x	x	x	x	x >	MS2										
Task 2.2 Presence of zoonotic parasites in fishery product imports on European key markets: case studies	x	x	x x	x	x	x	x	x x	x	x	x x	x	x	x x	x	x	x	x >	MS2										
Task 2.3 Presence of zoonotic parasites in Vietnamese Pangasius production systems.	x	x	x x	x	x	x	x	ı x	x	x	x x	x	x	x D2.2	x	x	x	x o	MS2										
WP3: SAMPLE & DATA MANAGEMENT																													
Task 3.1. Parasite sample management	x	x	D3.2 X	×	MS4	MS5	x	x x	x	x	x x	x	x	x x	x	x	x	x	ι x	x	x	×	xx	¢ X	x	x	x	x x	D3.1 D3.4
Task 3.2 Epidemiological sample management	x	x	MS3 x	x	x	x	x	x	x	x	x x	x	x	x x	x	x	x	x	ι x	x	x	×	x	x x	×	x	x	x x	D3.3
WP4: HAZARD IDENTIFICATION																													
Task 4.1 Molecular characterization and genetic structure of anisakids pbased on mtDNA cox2 gene			D4.1 x	x	x	x	x >	ı x	x	x	x x	x	x	x x	x	x	x	x )	c x	x	x	×	x	x x	×	x	x C	D4.2	
Task 4.2. Genetic indetification to the species level of anisakid nematodes by MAE			x x	x	x	x	x >	x	x	x	x x	x	x	x x	x	x	x	x o	ι x	x	x	×	x	x x	×	x	x C	D4.2	
Task 4.3 Developing of new and innovative nuclear markers obtained from microsatellites loci (SS-DNA loci) in species of the genus Anisakis.			x x	x	x	x	x	x	x	x	x x	x	MS6	x x	x	x	x	x o	c x	×	×	×	x	x x	×	x	x D	D4.2	1
Task 4.4. Genetic/molecular identification of parasites of zoonotic importance (other than anisakids) recovered in fish from the Asiatic region.				x	x	x	x >	x	x	x	x x	x	x	x x	x	x	x	x >	ι x	x	x	×	x	x x	×	x	x C	D4.2	
Task 4.5. Statistical analysis of the genetic data								×	x	x								x o	x x							×	x D	D4.2	
WP5: HAZARD CHARACTERIZATION																													
Task 5.1 Antigen characterization for parasites other than Anisakis spp								x	x	x	x x	x	x	x x	x	x	x	x o	t X	x	x	×	x	x D5.3	2 ×	x	x	x x	MS8
Task 5.2 Antigen exposure (mapping of allergens) for Anisakis spp. in fishery products				x	x	x	x >	x	x	x	x x	x	x	x x	x	x	x	05.1 )	ι x	x	MS7								
Task 5.3 Antigen proteomics, including genetic variability								×	x	x	x x	x	×	x x	x	x	x	x >	ι x	×	x	×	x	x D5.3	3 ×	x	x	x x	MS9
Task 5.4 Characterization of the immune response to the parasite antigens										x	x x	x	x	x x	x	x	x	x o	ι x	x	x	×	x	k X	×	x	D5.4	x x	MS8
WP6: IMPROVEMENT OF DETECTION METHODS FOR THE INDUSTRY AND OTHER END-USERS																													
Task 6.1. Evaluation of the mandatory visual inspection scheme for detection of parasites of human health significance in fish fillets. Development of a new technical device to test the viability of parasites in processed fish products	×	x	x x	x	×	x	x>	x	x	x	x x	x	×	x x	×	×	x	x	c x										
Task 6. 2. Technological enhancement of the UV-Press method for mass screening of parasites in fishery products	x	x	x x	x	x	x	x >	x x	x	xx	x x	x	x	x D6.2	x	x	x	x o	t x	x	MS10	x	x	x x					
Task 6. 3. Implementation of molecular methodology based on Real Time-PCR to detect parasites and/or their traces in fishery products.	x	x	x x	x	x	x	x	ı x	x	x	x x	x	x	x x	x	x	x	x o	D6.3										
Task 6. 4. Development of immune assays to detect parasites and/or their traces in fishery products.	x	x	x x	x	x	x	x	ı x	x	x	x x	x	x	x x	x	x	x	x o	t X	×	x	×	x	x x	×	x	x	x x	D6.4
Task 6. 5. Validation of the developed and/or implemented methods and evaluation of their performance by Ring Trials.																			×	×	x	×	x	x x	×	x	x	x x	×
Task 6. 6. Beta-testing of validated detection methods at industrial level																								x	×	x	x		
WP7: INTERVENTIONS IN THE FOOD CHAIN TO REDUCE RISKS																													
Task 7.1 Variation of the viability and infectivity of parasites in fishery products	x	x	x x	x	x	x	x	x	x	x	x x	x	x	x x	x	D7.1								MS1	1		M	IS12 x	D.7.6
Task 7.2 Interactions between parasites and bacteria of post harvest fish and their different storage conditions	x	x	x x	x	x	x	x	x	x	x	x x	x	x	x x	x	x	x	x	D.7.2					MS1	1		M	AS12 x	D.7.6
Task 7.3. Treatments for inactivation of Anisakids in fishery products	x	x	x x	x	x	x	x >	x x	x	x	x x	x	x	x x	x	x	x	x	ι x	x	x	×	x	x MS1	1 x	D.7.3	M	AS12 x	D.7.6
Task 7.4. Development of antigen elimination or inactivation methods	x	x	x x	x	x	x	x	x x	x	x	x x	x	x	x x	x	x	x	x	ι x	x	x	×	x	x MS1	1 x	D.7.4	M	AS12 x	D.7.6
Task 7.5, Technological enhancement of a device to kill zoonotic anisakids in discarding offalls onboard	x	x	x x	x	x	x	x	x x	x	x	x x	x	x	x x	x	x	x	x	t X	x	x	×	x	x MS1	1 x	D.7.5	M	AS12 x	D.7.6

#### Task 8.1. Statistical modelling and inference x x x MS14 Task 8.2 Evaluate prevalence of human health impacts of parasites in seafood x 191 Task 8.3 Quantitative risk assessment D8.2 Task 8.4. Analysis of willingness to pay D8.3 Task 8.5. Cost/Benefit analysis D8.4 Task 9.1. Catalogue/Portfolio of relevant technological solutions (tools, methods, devices...) D9.3 Task 9.2. Technology transfer supporting workshops. D9.2 Task 9.3. Roadmapping future prospects. Task 9.4 Development of communication materials by considering the characteristics of main target groups for the actions. Task. 9.5. Work with the media professionals Task 9.6. Implementation of communication and dissemination plan D9.

#### PARASITE 312068



**B1.3.3 Graphical Presentation of Work Flow** 

Fig. 3: Project work flow. Pert diagram

## 1.3.4. Risks and associated contingency plan

Risk	Countermeasures
Required fish samples not available in general or in the required amount	The probability of this occurring is low. Fishing SMEs in the project and access to samples from ongoing fish survey programmes guarantee access to the material needed. If necessary, appropriate replacements can be found because host species are replicated communities for parasites having zoonotic and public health implications. These parasites present a wide host range and specificity. In that case, more emphasis will be put on other samples of equivalent importance from an ecological, economic and/or medical point of view.
Partner bankrupt	If an organization has to withdraw from the project or ceases to exist, the consortium will propose redistribution of the work among existing partners or (where this is not possible or insufficient) suggest alternative partners to the EC. All partners in the Parasite Consortium are part of networks where finding alternatives for a specific partner should be possible without causing major inconveniences to the consortium and workplan. If required the advice of the Project Officer will be requested.
Non- or under-performing	The Consortium agreement will set up the measures that can be taken
partners Delays	by the Consortium members with the approval of the EC Some delay in achieving the listed deliverables and milestones could occur due to some technical difficulties, such as in getting sampling or to set-up and apply the methodologies, which are necessary to carry out other tasks-WPs of the project. However, any possible delay will be first communicated to the WP Leader and then to the Coordinator. Again, the strong and good collaboration between the Partners and the SMEs as well will help in managing in the best way and without extra amount of resources will guarantee the solution. The Project coordinator will continuously monitor the status of the project, supported by the WP leaders and the Steering Committee, and if needed, as a last resort the consortium could seek a non-funded extension to the project
Disagreements between partners	The management strategy has established the decision levels in the project. When the consortium agreement is negotiated, it will include the voting rules for the decision-making process, and this will set up a solution for the event of a tie, lack of quorum, etc. Most of the Participants in the Consortium have already established a long-term collaboration on anisakid nematodes and fish parasites. Therefore, any aspect related to that will be easily solved.
Amount or quality of work does not comply with the expectations	A partner may not fulfil his commitments. The Project Manager will inform the partner about the problem and its impact on the overall project performance. If the partner fails to respond positively, the Project Manager and the Steering Committee will try to solve the problems based on the Consortium Agreement. Indeed, the long-term collaboration between the participants will be able to solve the problems, for instance establishing a strong network between the laboratories performing similar tasks, including the possibility of training and exchanging of the personnel involved in the Consortium. Generally, all the participants of the Consortium are ready to help each others in order to solve any difficulty will arise during the implementation of the project. The multidisciplinary expertise of the Consortium will permit to obtain such solutions

## **B2.** Implementation

## **B2.1 Management Structure and Procedures**

## B2.1.1 Management structure and decision-making structure

The number and diversity of partners in the consortium provides the intended multidisciplinary and pan-european approach but, on the other hand, brings complexity to the project management. Furthermore, the ambitious proposed work-plan makes necessary to undertake an exhaustive management strategy, which allows a detailed monitoring of the activities foreseen, in order to detect any deviation and take the appropriate measures to correct it. It will also contribute to an adequate information flow, reinforcing the involvement of the SME partners and contributing this way to guarantee the expected impact.

The consortium and work plan in this project have been designed taking into account two aspects of special relevance, and with a strong influence in the project management structure and decision-making process. One of them is the research approach that should be undertaken, clearly targeted to SMEs' interests and needs. The other aspect is related to the European added value that the proposal should provide aimed to strengthen the competitiveness of European food producers. Both elements have been incorporated in the designed strategy by stressing certain measures which can contribute to a strong involvement of the industry, not only in the project technical development but also in the monitoring and decision-making processes.

So, according to the established in WP1, this set of activities is aimed to perform an operative project management by implementing the most appropriate tools and means that will guarantee a fluent exchange of information and an efficient and transparent decision-making process, including IPR issues. It also aims to assure an efficient project reporting and a satisfactory accomplishment and follow-up of all project items, especially milestones and deliverables.

A <u>Project Procedure Manual</u> will be issued at the very beginning of the project (month 2) for the partners' awareness of the internal communication and reporting rules as well as decision-making procedures.

#### Project management structure and decision-making process

For guaranteeing the adequate development of the work and the achievement of the project objectives, the management structure will be set up as described hereunder.

Participation of SMEs is guaranteed in all decision-taking levels. Furthermore, this participation will be reinforced by the voting rules and quorum criteria, which will be set up in a way that ensures their involvement in any relevant decision-making process. These matters are included in the Consortium Agreement.

CSIC-IIM is the partner coordinating the project and Dr. Santiago Pascual the person in charge of this work. LARPRO, led by Christian Larsson, will support this role acting as a <u>Project Secretariat</u> for the coordinator and the consortium members, particularly by carrying out administrative tasks related to meeting organization, reporting processes, management tools implementation and support, etc.

## Steering Committee

The Steering Committee (SC) will be the <u>executive body of the consortium</u>, consisting of the WP leaders, chaired by the Project Coordinator. Additionally, a representant of SMEs will assume a sixmonth rotating participation occurring simultaneously within each Project Assembly meeting. This will allow a best-value for money for SMEs that can be representing in the meeting points in their own country.

• Function:

The SC will be responsible for the day-to-day technical development of the project, including the work-plan follow-up and the application of the foreseen contingency measures, in case deviations are detected. For this purpose, six-monthly internal synthetic periodic reports, both technical and financial, will be issued and used for SC discussion and for preparation of project Assembly meetings. Such reports will be a useful tool for an early detection of any relevant deviations or problems affecting the project accomplishment and therefore for adopting decisions to avoid or minimize the related risks.

• Reporting:

The SC reports to the Project Assembly with regard to the technical decisions taken, according to its responsibilities.

## Project Assembly

The Project Assembly (PA), composed of one representative from each partner and chaired by the Project Coordinator, will be the <u>governing body</u> of the consortium. It will convene every six months.

• Function:

The PA will hold the ultimate responsibility for decision-making within the project and will decide on overall project issues not foreseen to be managed otherwise. Decisions of the PA are binding for all partners.

• Reporting:

The PA reports to the Commission through the Project Coordinator, by means of the periodic reports issued and providing any additional information requested by the project officers.

## Exploitation and Dissemination Committee

The Exploitation and Dissemination Committee (EDC), composed by partners owning the foreground of PARASITE exploitable results and responsible for exploitable deliverables, partner SMEs and, when suitable, external advisors and chaired by the Project Coordinator, will be the <u>communication</u> <u>branch</u> of the project. An IPR Advisory Group will be appointed from the members of this group.

External advisors are representatives from industry, consumer organisations and/or policymakers willing to follow up the progress in the project to reach the expected results and to facilitate paths for the exploitation of such results. Advisors will be identified by consortium members on a result-by-result bases, so that in each case it will be selected the most appropriate person to invite.

• Function:

The EDC will keep under review the project results, will provide advice on protection when appropriate and act as a link among partners to ensure that dissemination of results can be carried out safe and effectively, according to the work-plan. Bearing in mind the benefits of an early dissemination of the results achieved, due to the relevance of the tackled issues, the project <u>Communication Plan becomes a key tool</u>, it must provide orientation to partners to carefully select the information to be disclosed and the target public to which it is addressed. As the PARASITE proposal is strongly SME targeted and aimed to strengthen the competitiveness of European food producers, the participation of SME partners in this committee will be especially relevant. Their point of view will be extremely valuable to focus

the communication and knowledge transfer strategy towards those issues of particular interest for the industry.

Reporting

The deliberations of the EDC will be made available to all participants in the project through the Project Coordinator, on a six-monthly basis.



Figure 4. Project Management Structure

## Detection of deviations: Contingency Plan

Besides the above mentioned six-monthly internal reports, the time schedule for the project, together with the deliverables and milestones sets, will be the main items to be monitored to assess the project progress. Before each Project Assembly meeting, the Steering Committee members will carry out a simple check of them, which result will be presented for discussion and adoption of corrective measures if necessary, according to the Contingency Plan (see *Section B.1.3*).

The partner responsible for the task and/or WP affected will assess the effectiveness of the measures taken.

## Conflict resolution

Conflicts or problems of different nature will arise during the execution of the project. When they arise, they will be resolved according to the following principles:

- They will first be addressed within the relevant WP through discussion chaired by the WP leader.
- If no satisfactory solution is defined, the issue will be presented by the WP leader to the Project Coordinator who will analyze the problem and decide if an direct solution can be addressed or if the issue will be presented to the Steering Committee, depending on it's nature (scientific/technical or business/strategic), and severity. The Steering Committee will attempt to resolve the issue through a simple majority vote.

Any conflicts of a severe nature that cannot be resolved through the principles above, will be presented to the Project Assembly, and if necessary even to the European Commission according to the dispute resolution provision set forth in the Consortium Agreement.







Figure 6. Problem solving flow chart

## IPR management

The research results arising from the project (Foreground Intellectual Property – IP) will be property of the partner(s) who have produced them. The IPR management will be dealt within the Consortium Agreement, which will be concluded among all participants prior to the project start.

IPR issues will be under the responsibility of the IPR Advisory Group and the Exploitation and Dissemination Committee.

## **Consortium Agreement**

The Coordinator, taking into account partners' instructions and suggestions, will prepare and propose a Consortium Agreement. It should be signed and submitted to the Commission before the entry in force of the Grant Agreement.

This document is meant to address in detail the general governing rules within the consortium during the project duration, regarding to issues such as internal organisation, management of the EC financial contribution, rules on foreground dissemination and use, including IPR management, voting procedures for decision-making, settlement of internal disputes, etc.

## Communication among partners

The Project Coordinator has the duty and the commitment of encouraging a permanent communication flow within the consortium, which is essential for all partners to play their role and for the adequate development of the work-plan. Also, a suitable and well-managed internal communication will be of advantage to boost the role of SMEs in the project by encouraging and facilitating their involvement.

For this purpose, <u>e-mailing lists</u> in accordance with the different work-packages and decision levels in the project will be set up.

On the other hand, in order to facilitate the document and knowledge management, a <u>project intranet</u> will be implemented. Available commercial and free software will be taken into account before the Steering Committee makes a proposal to the Project Assembly about the ICT tools to be used for project management. The chosen tool should fulfil cost, suitability and user-friendliness criteria, given that it must add value to the project management and it should not increase the workload but, on the contrary, lighten it.

## Communication and reporting to the EC

The Steering Committee, through the Project Coordinator and with the support of the whole consortium, will be responsible to gather and collate all the information and documents from partners, in order to prepare the required reports and submit them in due time.

The Coordinator will be the sole interlocutor with the Commission, being at its disposal for any review or hearing about the activities planned or being carried out, as well as transmitting any questions arisen within the Consortium, during the project life-time.

## Meetings and internal workprogress monitoring

Meetings are essential for coordination and for workprogress monitoring; in short, for a right development and the achievement of the planned results and therefore the expected impact. By setting and fulfilling a periodic meeting agenda, the Consortium will be able to:

- keep under control the risk of potential deviations from the work-plan;
- minimise the risk of expected results not being achieved, by taking early measures when deviations are detected;
- foster a strong involvement of SME partners in the project;
- guarantee a fluent and suitable communication of results, particularly with regard to technology transfer towards industry;

- share out-of-schedule dissemination activities foreseen and/or carried out and assessing their convenience and impact;
- favour the interaction among partners for boosting cooperation.

Project Assembly meetings will be held in a six-month basis, following a kick-off meeting at the very beginning (month 1). The Steering Committee will meet within one month in advance, either in person or by videoconference, in order to prepare the necessary interim reports to be shared with the Consortium during the PA meetings.

Extraordinary unforeseen meetings could be also scheduled, if necessary.

In order to optimise the project resources and to avoid an overload of travel time, videoconference facilities will be used for multi or bilateral meetings when appropriate, at any moment during the project lifetime. This option will specially be considered for the involvement of Asiatic partners in project meetings.

The meeting agenda will be updated by trying to match different project activities within the same time framework.

MEETING	Молтн	PLACE	MEETING	Молтн	PLACE
Kick-off meet.	1	Spain (Vigo)	5 <sup>th</sup> meeting	24	Germany
2 <sup>nd</sup> meeting	6	Norway	6 <sup>th</sup> meeting	28	UK
3 <sup>rd</sup> meeting	12	Italy	Final meeting	36	Spain (Vigo)
4 <sup>th</sup> meeting	18	Belgium (Brussels)			

## **Meeting Schedule**

## Methodology for meetings organization

Each of the scheduled meetings will be held in one partner country, as shown in the table above. For a greater efficiency, meetings will be tried to match with some planned dissemination activity, so most of the project partners could participate in both. Furthermore, the following considerations will be of particular relevance for the preparation of meetings:

- The meeting agenda will be agreed and shared in advance within the Consortium;
- The Steering Committee will make the previous work of gathering information on work progress and discuss the information obtained and identify key issues to be treated in assembly meetings.
- The agenda will always include specific sessions for IPR and dissemination activities discussion;
- The minutes will be written by the Project Secretariat and made available to all partners, by email and at the intranet, and to the Commission (or REA) through the SESAM facility.
- Assigned Project Officer will be informed about meeting plans and invited to attend.

## **B2.2 Beneficiaries-Individual participants**

# *B2.2.1 PARTNER 1 AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS, CSIC, SPAIN*

## Description of participant

The Spanish National Research Council (CSIC) is the largest public institution dedicated to research in Spain and the third largest in Europe. Four research groups belonging to three research centers participate in the PARASITE project:

## **CSIC - INSTITUTO DE INVESTIGACIONES MARINAS (IIM)**

It is a multidisciplinary research institution with widely recognized experience in several research lines in marine science such as Natural Resources and Food Science and Technology and engineering. IIM comprise four departments: Ecology and Marine Resources, Oceanography, Biotechnology and Aquaculture, and Food Technology.

## CSIC - INSTITUTO DE CIENCIA Y TECNOLOGIA DE ALIMENTOS Y NUTRICIÓN (ICTAN)

ICTAN works in different areas of Research and Development in Food Science and Technology. At present the ICTAN has 3 departments based on horizontal disciplines (Food Quality and Safety, Metabolism and Nutrition, Process design and development) based on food products in which Fish and Fish Products is one of the main research areas. **CSIC - MUSEO NACIONAL DE CIENCIAS NATURALES (MNCN)** 

The MNCN is the most important scientific institution in Spain dedicated to research, conservation and communication of the Natural History, and it is a national and international reference point on this subject. Research activities in MNCN are organised in five Departments: Evolutive ecology, Biodiversity, Environmental Biology, Geology and Paleobiology.

## **Attributed Tasks**

CSIC will be the Coordinator of PARASITE, and the leader of WPs 1, 3, and 7. CSIC will participate in all WPs providing knowledge and experience in the field of sampling and data management, epidemiology, detection and mitigation methods and technologies, parasite biology, genetics, proteomics and dissemination.

All CSIC researchers have a wide experience in different areas related to the PARASITE project. All of them publish in mainstream scientific journals and have a wide background in participating and coordinating European, National and Regional RTD projects. All the groups also cooperate and work in strong relationship with the Industry.

## Research groups relevant experience

## CSIC (IIM-E)

The main goal of the Research Group Ecology and Marine Biodiversity (Ecobiomar) is the study of biodiversity, community structure and abiotic and biotic interactions in marine ecosystems. Actually, this is concreted in two research fields: parasite and marine ecology. One of the issues derived from the main goal is the study of the spatial/temporal variability of natural resources in relation to atmospheric-oceanographic parameters obtained in oceanographic surveys and using satellite data for applying predictive models. We also deal with bottom-up and top-down control on recruitment, growth, ageing, reproduction and feeding habits of marine, as well as the interactions between preypredator and host-parasite systems worldwide. The ecology of parasites research line focus on the study of the most important potential pest species affecting fish populations with public health concern and on the development of devices and methods to minimize the zoonotic risk. The goal of the ecology of parasites is linked and focused, especially, to solve practice cases related to Ictiology and Malacology, as well as the emerging hazard due to parasites in fish of commercial interest reaching the EU markets.

## CSIC (IIM-QPM)

The Seafood Chemistry group deals with chemical and biochemical methods for the quality evaluation of fishery products; Assessment of chemical metabolites related to quality loss; Lipid oxidation in fishery products; Evaluation of the endogenous prooxidant

and antioxidant systems natural antioxidants employment; Application of proteomic technology in the species identification study; Origin control; Use of infrautilised fish species; Application of new technological strategies during fishery and farmed species chilling.

## CSIC (ICTAN)

The Group participating in the proposal has a solid experience on Food Science and Technology focussed on fish and fish products from different aspects. The group has been involved in research in new methodology to evaluate fish quality, safety, and authenticity. It is engaged with the application of spectroscopy (i.e. vibrational, NMR, time domain reflectometry) to analyze fish muscle components. It has demonstrated experience in authentication including species, geographical origin, or time/temperature history of frozen fish. Since 2006 the group is coordinating projects financed by the Spanish R&D National Plan concerning *Anisakis* infecting fish. The group has led the research projects on the effectiveness of different technological and culinary treatments in mortality of Anisakidae larvae and their effect on the immunogenicity of allergens from the parasite, working together with immunology experts from the Hospital Carlos III (leaders of WP4 in this proposal) and the ongoing project Detection and identification. Selective treatments applied and effect on the allergens from different *Anisakis* sp and origin (ANIDET project) together with the Hospital Carlos III and expert parasitologists from the CSIC-MNCN

## CSIC MNCN

The scientific aim of the Biodiversity and Evolutive Biology Department is to describe biodiversity patterns in animal and to infere and demosntrate the mechanisms and proccesses linked to them under an evolutive perspective. The research lines comprises different levels, including taxonomical description and molecular analysis that allow phylogenetic relationships from biogeographic patterns to demography, behaviour, interaction, etc. Nematodes are one of the target species studied by this research group.

#### Staff member profiles CSIC IIM-E

**Dr. Santiago Pascual**, Project Coordinator is currently a Tenure Scientist of CSIC. He has published more than 80 papers within the SCI of research on the ecology of marine parasites and parasite-induced pathology in marine organisms. Multidisciplinary studies on disease epidemiology have been a speciality including topics from marine contamination to biodiversity and fisheries management. He is currently leading a Spanish network devoted to translate the research on parasites to the fishing industry and official inspectors involved in the management of this biological hazard in the food chain.

**Dr. Ángel F. González**. Scientific Researcher of CSIC. Head of the Marine Resources and Ecology Department and of the Marine Ecology and Biodiversity Research Group. He has participated in 27 research projects on Marine Ecology. He has published 70 papers on SCI journals. Lecturer of postgraduate courses in Marine Biology and Fisheries Management at three Spanish universities. Member of the Editorial Board of Fisheries Research and referee of 17 international journals.

**Prof. Angel Guerra**. Research Professor of CSIC. He leaded more than 20 research projects. About 200 scientific publications within SCI mainstream journals, book chapters and books. Lecturer of postgraduate courses in Marine Biology and Fisheries Management at three Spanish universities. Supervisor of 15 Ph.D. theses. Ex-President of the Spanish Marine Research Centre and member of the Editorial Board of Fisheries Research.

**Manuel García Blanco** is a degree in Biology, Senior Technical Sampling Manager at the IIM-CSIC. He has developed his professional career in the Spanish Oceanographic Institute (IEO), always within the field of providing sample and data management of many different components of the marine ecosystem. It also participates actively in various fish sampling programmes in National and EU projects.

A degree in Veterinary with demonstrated skills in Parasitology and Food safety will be contracted to undergo tasks related with diagnostic tools and self-monitoring analysis and reporting technology.

**CSIC IIM-QPM Prof. José M. Gallardo**: Full Professor at CSIC and together with Dr. Medina, leadership of the group of Seafood Chemistry. He performed his PhD degree in Chemistry in Santiago de Compostela University (1972). He is a Proteomics expert and has been the coordinator of several national financed programs aimed to the identification of marine proteins by mass spectrometry. He has published more than 100 peer reviewed papers and received some awards for their contributions to Marine Proteomics.

**Prof. Isabel Medina**: PhD. in Chemistry 1995, Santiago de Compostela University and Full Professor of CSIC. She has a wide expertise in marine lipid and protein analysis. She has published more than 80 peer review papers and is author of 8 patents, the most of them licensed to industry. In the last 10 years, the group has developed a high expertise on the application of Proteomics in different areas of seafood science, as the determination of allergenic proteins, analysis of specific proteins and peptides for species identification and studies of proteome oxidation in tissues.

a degree in Chemistry, Biology or Marine Sciences will be contracted to assist with monitoring of specific peptide biomarkers using 2-DE analysis, MALDI-TOF MS and nESI-IT-MS.

## CSIC ICTAN

**Prof. Margarita Tejada** is a Research Professor of CSIC. The area of work is food science and technology. Member of the International Institute of Refrigeration, and of the West European Fish Technologists Association (WEFTA). Since 2005 is coordinator and project leader of several projects in parasites focused in the effectiveness of different technological and culinary treatments in mortality of Anisakidae larvae and their effect on the immunogenicity of allergens from the L3 larvae, and in the development of techniques for evaluating allergens from different Anisakidae that infect fish muscle.

**Prof. Mercedes Careche**, Research Professor at CSIC. Her research lines include the development of tools for the evaluation and improvement of the quality and control of traceability of seafood; Development of sensory methods, and the application of multisensor techniques to evaluate fish quality; Elucidation of mechanisms responsible for quality loss in fish muscle: the relation between the structure, ultrastructure of the muscle and the technofunctional properties and texture attributes; Consumer oriented design and development of functional seafood products.

**Prof. M**<sup>a</sup> **Teresa Solas**, professor at the Cellullar Biology Department, Biology Faculty at the Universidad Complutense de Madrid (UCM) Spain, since 1985. She has collaborated in Spanish and UE projects, applying her expertise in Microscopy in the field of Food Science and technology, related to fresh, frozen and treated food, mainly fish and fish products. Since 2006 she is participating in projects related to *Anisakis* being in charge of the detection of the changes observed in the larvae and parasitized fish tissue and the detection of allergens in larvae and parasitized muscle by immuno-histochemistry (IHC) after storage and culinary treatments.

**Angel Mendizábal**, Head of the Technical Unit of Mercamadrid, which is after Tokyo Fish Market, the largest Fish Market in the world. He participated in the research projects of R&D Spanish National Plan dealing with the effect of treatment on the recognition of allergens.

A post doc position will be contracted for studies of the effectiveness of the physical chemical treatments given to the fish to kill the parasites, as well as the effect of these treatments on the quality of the fish products. These will include the study of the sensory attributes and physical chemical analyses related to the quality of the fish, as well as monitoring changes in components using vibrational spectroscopy and TD NMR spectroscopy.

## CSIC MNCN

**Dr. Alfonso Navas** is Senior Researcher of CSIC. He has been Director of the Museo Nacional de Ciencias Naturales. Member of the Governing Board of the European Society of Nematology 1996-2000. Head of the Fauna Europaea End Users Committee. Representative of CSIC at the European Thematic Centre (Paris) of the European Environment Agency Member (2002-2005). His research lines have been included

basically in Zoology into Nematology. His work has been developed in Systematics and Taxonomy of Nematodes, Ecology, Epidemiology, Genetics and Evolution. Currently is working in several projects on Proteomics application to Phylogenetics and Evolution and leads the National Laboratory of Reference for Plant Parasitic Nematodes.

**Dr. Lee Robertson** has several years experience in research on important nematodes and nematodes of quarantine concern in addition to those of economical importance. He has more than 30 peer reviewed scientific articles. His activities include molecular and biochemical characterization of the host parasite interactions of important nematodes. He has taken part in aspects of the EU directive relating to parasitic nematodes of the genus Globodera. He is involved in the sequencing of the *Anisakis simplex* and *A. pegreffi* genomes and the transcriptome analysis of both species.

**Dr. Susana Cobacho Arcos** is Nematode taxonomist and is currently carrying out a project on Phylogeny and Evolution of Enoplida and Cromodorida incorporating molecular and proteomics tools in and integrative taxonomy. In addition she is also working on bacteria-nematode interactions as model for studying influences of bacterial infections in population and species differentiation of nematodes.

It is foreseen to contract an expert on Nematology (at Ph degree level) with skills in Taxonomy and Proteomics of parasite nematodes. Under Projects needs, it would be considered also the contract of a technician for experimentation's support.

## B2.2.2 PARTNER 2 NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH NASJONALT INSTITUTT FOR ERNÆRINGS-OG SJØMATFORSKNING, NIFES, NORWAY Description of participant

The **National Institute of Nutrition and Seafood Research (NIFES)** is a research institute owned by, and directly linked to, the Norwegian Ministry of Fisheries and Coastal Affairs and gives research based advice to the government and national food safety authority in matters concerning the "fjord to fork" production chain of seafood, as well as scientifically supporting the Norwegian fisheries industries. Additionally, NIFES acts as the national reference laboratory for several seafood safety issues including parasites of quality reducing and human health concern in fish and fishery products. The parasitological research and advisory activities at NIFES are currently organised under the institute's seafood safety research program, facilitating interdisciplinary research, e.g. at the interface between Parasitology and microbiology. To support the various research and monitoring activities, NIFES enjoys a range of state-of-the-art laboratory facilities, including advanced microscopic equipment and various microbiological, immunological and molecular techniques.

## Attributed Tasks

NIFES is the leader of WP2, and will participate in WP3 and WPs 6, 7, 8 and 9.

#### Relevant experience

As part of our parasitological activities and obligations, NIFES runs since 2006 a long-term surveillance program on quality reducing and/or potentially human pathogenic parasites and bacteria in various commercially important pelagic fish stocks including Atlantic mackerel, herring and blue whiting, from the main fishing grounds in the North- and Norwegian Sea. The results of the surveillance program, which is based on a mandate from the Norwegian food safety authority (NFSA), are regularly reported to the NFSA or – upon request – to other food safety authorities or advisory bodies such as EFSA and ICES, respectively. Another important aspect of our activities is related to the enhancement or adjustments of actual detection methods for efficient and accurate parasite recording in fish under field or industrial conditions.

## Staff member profiles

**Dr. scient. Arne Levsen** holds since 2002 a senior scientist position at NIFES. He is in charge of the Parasitology and immunology research which is integrated in the institute's seafood safety research program. Dr Levsen is currently running or involved in several research projects, e.g. funded by the Norwegian research council (NRC) and the NFSA, which address various parasitological aspects concerned with the safety and quality of fishery products. His current research focus is on the evolution of life cycles in marine

parasites, especially as to phenotypic and genotypic plasticity, using the nematode *Anisakis simplex* (s.s.) as a model organism. In another cooperative project he investigates the taxonomy of the myxosporean species *Kudoa thyrsites* in Atlantic mackerel using both morphological and molecular techniques. Dr Levsen has since 2007 been an attending deputy member of the National committee for contagious diseases from food. During 2009-2010, and in 2011, he was a scientific expert member of two EFSA working groups under the BIOHAZ Panel; 1) on 'Risk assessment of parasites in fishery products', and 2) on 'Fish parasites in the Baltic Sea'.

**Dr. scient. Bjørn Tore Lunestad** was employed by the Directorate of Fisheries in Bergen as head of the microbiological section of the Central Laboratory in 1992, and has since 2002 held a senior scientist position at NIFES. In 2006 he was employed as an Associate Professor in seafood microbiology at the Department of Biology, University of Bergen. During several externally financed projects, his scientific activities have mainly focused on quality reducing and human pathogenic microorganisms in seafood. He has since 2004 been a member of the National scientific committee for food safety, group on hygiene and contagious agents, and since 2007 a selected member of the National committee for contagious diseases from food. Dr Lunestad has participated in several EU and CODEX working groups related to seafood safety.

**M.Sc. Cecilie Smith Svanevik** received her Master-degree in food microbiology in December 2010 with a thesis on the bacterial flora of Atlantic mackerel. She has since been working as a scientist at NIFES under the seafood safety research program. Mrs Svanevik is p.t. mainly involved in the surveillance program on parasites and bacteria in pelagic fish where she is responsible for data collection and analysis with regards to general quality assessments and various hygiene parameters.

## B2.2.3 PARTNER 3 TUSCIA UNIVERSITY (UT-URS) ITALY

## Description of participant

The **Tuscia University (UT-URS)** with the Department of Ecological and Biological Sciences. It aims to bringing together researchers, infrastructure and skills concerning environmental assessment, evolutionary issues and bio-medical areas. The Laboratories of the Department involved in the Project proposal will be: the Laboratory of Molecular Ecology and the Laboratory of Parasites Ecology, created in collaboration with the "Sapienza-University of Rome" (URS). The Laboratory of Molecular Ecology support studies on DNA variation using a variety of genetic/molecular approaches. The Laboratory of Parasites Ecology and ecology of parasites of marine and freshwater organisms. The Lab is also linked to the Marine infrastructure Centre for Experimental Aquaculture at the Saline of Tarquinia (CISMAR), belonging to the same Department of Tuscia University.

## Attributed Tasks

UT-URS is Leader of WP4; Participant of: WP2, WP3, WP6, WP8, and WP9

## Relevant experience

Tuscia University has a long-term collaboration with Sapienza-University in Rome to carry out research on molecular systematics, population genetics, ecology, host-parasite co-evolutionary aspects, seafood safety and quality of parasites of various aquatic organisms (fish, marine mammals, fish-eating birds), particularly on anisakid nematodes.

#### Staff member profiles

**Simonetta Mattiucci** senior Research at the Department of Public Health Sciences and Infectious Diseases of "Sapienza-University of Rome" (URS). She is Aggregate Professor of parasitology in the Faculty of Medicine and Pharmacy of URS; contract Professor of "Marine Ecology" and "parasitology" at Tuscia University and in the Doctorate School of "Ecology and conservation of Biological resources. She teaches in the Veterinary Master Degree on Food Inspection of Animal Origin. Since 2007 she is the Chair of the

International Association of Fish parasitology. On 2011, she was a scientific expert member of the EFSA working group under the BIOHAZ Panel on 'Fish parasites in the Baltic Sea". More than 25 years of such researches have been dedicated to anisakid nematodes, especially related to Molecular Ecology.

*Giuseppe Nascetti*. Full Professor of "Fundamental Ecology" at the Department of Ecological and Biological Sciences (Tuscia University). He is responsible of the laboratory of Molecular Ecology of the same Dep. He is presently "Vice-Chancellor" of the Tuscia University. The main research interest concerns the molecular ecology and evolution, and the main topics include: host parasite-coevolution, genetic diversity at the inter- and intrapopulation level, genotype-environment relationships, speciation mechanisms, phylogeography and conservation genetics of fish, amphibians, and parasites of aquatic organisms.

**PhD Michela Paoletti.** Full Degree in Biological Sciences. Post-Docposition at *Department* of Ecological and Biological Sciences of Tuscia University. Molecular systematics of parasites of aquatic organisms, mainly on anisakid nematodes. Skilled experience in PCR-DNA genetic/molecular methodologies, including sequencing of several genes, statistical analysis of genetic data. She is co-Author of several papers concerning anisakid nematodes in peer reviewed Journals (Parasitology field).

**PhD Paolo Cipriani.** Full Degree in Environmental Sciences. Post-Doc position at *Department of Ecological and Biological Sciences* of Tuscia University. Molecular systematics of several parasites of aquatic organisms. Skilled experience on allozymes (MAE) methodology and Parasitological examination of fish, marine mammals, amphibians. Co-Author of papers in peer reviewed Journals (Parasitology field).

**PhD Mario Santoro.** Full Degree in Veterinary Medicine. Post-Doc position at *Department* of *Ecological and Biological Sciences* of Tuscia University. Skilled experience in parasites of aquatic organisms. He has published 20 papers in peer reviewed Journals and Book concerning parasites.

## **B2.2.4 PARTNER 4 AGENCE NATIONALE DE SECURITE SANITAIRE, ANSES, FRANCE** Description of participant

The laboratory for fishery products (about 20 persons) of the **French agency for food**, **environmental and occupational health and safety (ANSES)** has a significant experience in quality and safety of fishery products. It works in cooperation with seafood professionals and authorities to assess risks related to microbiological and biochemical quality of seafood products. It performs laboratory analyses in microbiology and chemistry for fish industry and health authorities and participates to research programs in the field of seafood quality and safety.

#### Attributed Tasks

## ANSES participates ion WPs 1, 2, 4 and 9

## Relevant experience

Parasitology is a new research orientation for this partner; its development was initiated with the recruitment of a junior scientist specialized in seafood Parasitology and microbiology and the willing of the French Food Safety Agency General Direction. Its main skills in Parasitology are knowledge on parasite biology and classification, detection techniques and use of molecular tools to identify and/or detect seafood pathogens. Worries related to fish parasites from authorities and professionals led to the setting up of this new activity in this laboratory, thus answering one of its institutional missions: assessment of health and nutritional benefits and risks.

Research developed is mainly supported by ANR (National Research Agency), France Agrimer (établissement national des produits de l'agriculture et de la mer), CPER (investment contract between state and regional authorities) and ARCIR (regional research support).

Regarding fish parasites, a research project, supported by a grant from the French Ministery of Food, Agriculture and Fisheries to asses anisakid prevalences in mackerel and

whiting was carried out from November 2009 to March 2011. This laboratory is also one of the major partners of a French project, funded by the National Research Agency and entitled "Fish Parasites: hazard identification, impact, and researches to define an efficient strategy of prevention". This project aims at improving the safety of fish and fish-derived products through a multidisciplinary work program that includes: (i) identifying larval nematodes or cestodes detected in the most currently consumed fishes in Europe; (ii) exploring the potentially structuring role of host species, geography, seasonality and other factors on parasite population; (iii) providing technical strategies to improve parasite detection in fish fillets; (iv) exploring the involvement of fish parasites in the alteration of marketable fish products; (v) setting a scientific platform (located at the ANSES laboratory) to help the operators (business operators, technicians, veterinaries and even staffs of fish stores) to identify parasites in fish; (vi) developing continuing education, training programs for staffs of industry or fish-stores, other professionals and specialized media staffs. This laboratory is developing strong relationships with the French National Reference Laboratory for parasites transmitted by feed, located at the ANSES Laboratory for Animal Health and with the French Directorate General for Food (DGAL).

#### Staff member profiles

**Melanie GAY** carried on undergraduate and graduate studies in Population and Ecosystem Biology, specialised in Parasitology. She received her PhD degree from University of La Rochelle in 2004 with a dissertation on oyster summer mortality, pathogenicity and taxonomy of Vibrio sp. isolated from oysters. Since 2005, she has set up ex nihilo the Parasitology activity in the laboratory for fishery products. Taking advantage of her experience in both microbiology and Parasitology, she is involved in 2 units of this laboratory: as a scientific researcher in microbiology (detection and identification of human pathogenic strains of Vibrio in seafood) and as the coordinator of the Parasitology activities. She has developed a strong network of scientific colleagues in the field of fish Parasitology leading to the acceptance of the funding of the project "Fish Parasites" by the French National Research Agency (ANR). She has developed skills in theoretical Parasitology, seafood safety, health hazards related to foodborne parasites, molecular biology and identification and/or detection tools for pathogens.

## B2.2.5 PARTNER 5 CENTRO TECNOLOGICO DEL MAR - FUNDACION CETMAR, SPAIN Description of participant

The **Centro Tecnológico del Mar, Fundación CETMAR** is a Public Foundation promoted by the Regional Government of Galicia together with the former Spanish Ministry of Science and Technology, currently the Spanish Ministry of Science and Innovation. CETMAR aims to improve the conditions for the sustainable use of marine resources and in this framework, to increase the efficiency of related economic sectors, namely the fishing industry, aquaculture and seafood processing industries, etc. It has a relevant background in coordinating collaborative research and innovation projects and networks under different types of funding schemes and programmes, in national, European and international ambits (Framework programme 6th and 7th, Life Programme, INTERREG, Third Countries' Development Programmes, etc.). In fact it has participated in more than 50 projects and initiatives co-funded under European Funding Programmes. In all of this project CETMAR has been or is playing a relevant role regarding networking of fisheries stakeholders (including industry, administration and academia), technology transfer, dissemination of RTD and Innovation activities and results, integration of R&D capacities, etc.

#### Attributed Tasks

Leader for WP9 and will participate in WPs 1, 6 and 8.

## Relevant experience

CETMAR will contribute to this project involving in it two of its departments: the Technology Promotion and Transfer Dept., and the Fish and Seafood Technologies Department. The background offered by involving these two areas in the PARASITE project covers

aspects such as: A) Expert knowledge regarding the presence of parasites in fish and seafood projects, and the consequences of this matter to the industry from a technical point of view. (TEDEPAD project; collaboration in the organisation of the International Symposium about Strategies to Manage Fish Parasites (Vigo 2010), WOPER SSA, EPISTOCK, MOLFISH, ANITECH. B) knowledge regarding technology transfer; C) knowledge in identifying technology gaps and in prospecting future technology challenges. (Technology foresight and watch activities and services, OATP project); D) Relevant experience regarding communication and dissemination strategies regarding technology and science and specially regarding fish and seafood hazards communication (As for example collaborations with administration bodies such as INTECMAR, AESAN and DG SANCO); E) Experience in designing and organising numerous internal and external training activities within the framework of many of the projects into which CETMAR and both of the departments have been involved. Furthermore CETMAR has also a training department that will provide eventual advice and support in case needed.

## Staff member profiles

**Ms. Rosa Fernández**: Head of technology promotion and transfer Department at CETMAR since 2002. Will act as contact person for the project at CETMAR. Degree in economics and an MBA by Caixanova Business School. Key background: technology transfer especially in fisheries and aquaculture, dissemination, innovation management, Framework programme project's management, technology foresight and technology watch. **PhD. Julio Maroto**. Head of Fish and Seafood Technologies Department at CETMAR since 2002. PhD in Biology (Zoology) (University Complutense of Madrid, 1992). Key background: hugely experienced in projects about Food safety and fishing by-products valorisation. High knowledge in private companies within the seafood processing industry after an 18-year-experience in production tasks as well as quality control and management.

**PhD Elvira Abollo**. PhD in Marine Parasitology (University of Vigo, 1999). High quality training and developed skills in sampling programmes, parasite diagnosis and biotechnology. Large experience in local, national and EU scientific projects dealing with parasite genomics and OIE listed diseases.

**Ms. María Pérez**: technical staff of technology promotion and transfer department at CETMAR since 2004. She has a degree in economics. Key background: technology transfer, especially in fisheries and aquaculture, innovation management, Framework programme project's management, technology foresight and technology watch.

## B2.2.6 PARTNER 6 SERVICO MADRILEÑO DE SALUDO, SERMAS, SPAIN

## Description of participant

**Servicio Madrileño de Salud (SERMAS)** is a public entity that manages all the public hospitals in the Madrid region, (including Hospital Carlos III, Hospital La Paz and Hospital de Toledo). SERMAS is assignated as a reference center for certain diseases, being the most characteristic activities in the health care and research areas: HIV treatment and research, tropical diseases, international adoption, international vaccination and Units for Amyotrophic Lateral Sclerosis and Traveler Medicine. These hospitals are present in national and international research in Infectious Disease, Immunology, Neurology and Internal Medicine.

SERMAS has the knowledge and infrastructure necessary for the determinations required for the study of humoral and cellular immune responses and molecular biology techniques. Furthermore, the Department of Immunology has a facility for *Anisakis* allergy diagnosis. The Department of Immunology comprises 1 Head of Department, 1 Chief of Section, 2 Immunology Specialists, 2 research fellows and 3 laboratory technicians.

#### Attributed Tasks

SERMAS will lead the WP 5, and it also will participate in WPs 3, 4, 7, 8 and 9. Relevant experience

The Department of Immunology at the HCIII carries out basic and clinical research mainly focused on type I hypersensitivity responses, with special interest in *Anisakis* and pine processionary moth allergies, and occupational asthma. Team members have been

publishing on different *Anisakis* allergy aspects since 2000. The impact of the results includes both clinical and basic aspects of *Anisakis* allergy. Among their contributions, it is worth noting the application of the flow cytometry for the *Anisakis* allergy diagnosis, the isolation and characterization of several parasite allergens, analysis of immune response related to the type of clinical symptoms in anisakidosis and analysis of antigenicity and allergenicity of parasite proteins after being subjected to several technological procedures. Therefore, the Department of Immunology members have a solid experience in approaching a public health issue such as *Anisakis* allergy. Furthermore, they are working with other experts on food and nutrition technology led by M. Tejada (ICTAN, Madrid) in order to analyze the effect of technological procedures on the parasite.

Ongoing research in the Department of Immunology includes national and international projects on pine processionary caterpillar allergy, animal allergy in animal facility workers and *Anisakis* allergy.

## Staff member profiles

**Dr. Miguel González Muñoz** degree in Veterinary and Degree in Biology. He has been working as a specialist in Immunology at the Hospital Carlos III (Madrid) since 1991. He has 47 publications in immunology area. Currently his work is focused on the characterization of type I hyerpersensitivity responses to different allergens. He has been working in *Anisakis spp.* allergy since 2005 and has published 10 articles on the characterization of specific immune response to *Anisakis spp.* He has a patent on detection of *Anisakis* antigens in food.

**Dr. Ignacio Moneo** obtained his MD in 1972, Zaragoza University, Spain. Internship in Bilbao, Spain in 1973, Residency in Immunology at the Clinica Puerta de Hierro, Madrid, Spain (1974-1977). From 1977 to 1990 he worked as staff member at the Immunology Department of the Hospital Ramon y Cajal, Madrid, Spain. Since 1990 he works as Head of the Department of Immunology of the Hospital Carlos III, Madrid, Spain. He has published 107 articles, among them 27 related to *Anisakis simplex* allergy, which is one of his areas of interest.

**Dr Álvaro Moreno-Ancillo** graduated in 1990 from Complutense University of Madrid, Spain, and he completed residency training in Allergology (1991-95) at the University Hospital La Paz of Madrid, Spain. His residency work has resulted in more than 10 technical publications in Anisakiasis (N Engl J Med, Ann Allergy Asthma Immunol, Allergy, J Gastroenterol Hepatol, Rev Esp Enferm Digest, Parasitol Res and J Investig Allergol Clin Immunol). He currently is a Clinical Allergologist at Hospital General Nuestra Señora del Prado Spain. His clinical interests are Anisakiasis, several asthma and food desensitization.

**Dr Jesús Jurado-Palomo** graduated in 2003 from University of Córdoba, Spain. In June 2008, he completed residency training in Allergology (2004-08) at the University Hospital La Paz of Madrid, Spain. He has recently published two articles in Anisakiasis (J Investig Allergol Clin Immunol). He later obtained his Graduate Expert in Clinical Genetics at the University of Alcalá de Henares, Spain (2008-09). He currently is a Clinical Allergologist at Hospital general Nuestra Señora del Prado, Spain. He has special interest in hereditary angio-oedema, *Anisakis* and genetic aplicated allergology

**Dra Carmen Panizo Bravo** graduated in 1986 from Autonomous University of Madrid, Spain, and she completed residency training in Allergology (1987-91) at the University Hospital La Princesa of Madrid, Spain. He currently is a Clinical Allergologist at Hospital General Nuestra Señora del Prado, Spain. She later obtained her Master's Degree in Bioethics at the Complutense University of Madrid, Spain (2004-06). She has special interest in immunotherapy and rhinitis

**Dr Santiago Quirce** is Head of the Allergy Department at Hospital Universitario La Paz, Universidad Autónoma of Madrid, Spain. He graduated in Medicine at the University of Santander, Spain, in 1983, and he obtained his Ph.D. degree at the University of Cantabria, Spain, in 1991. After completing specialty training in Allergology at Ramón y Cajal Hospital in Madrid, he spent one year as Research Fellow at Vancouver General Hospital, Respiratory Division, University of British Columbia, Canada. Then he joined

Fundación Jiménez Díaz at Madrid, where he spent 9 years as a consultant allergist and clinical researcher.

He has a broad research interest including asthma, rhinitis and occupational allergy. He is a member of CIBERES (Spanish Research Network on Respiratory diseases) and has ongoing research projects with NIOSH (National Institute of Occupational Safety and Health, USA) on the genetic of occupational asthma, and has participated as a researcher in the EUROPREVALL (The prevalence, cost and basis of food allergy across Europe (Europrevall). Subproject: Pediatric allergy, PI-2119 from the European Commission). Dr. Quirce has edited and authored numerous publications, including 4 books and over 200 peer-reviewed papers. He is a member of several Spanish and international scientific societies and he is currently member of the board of Occupational Allergy of the European Academy of Allergy and Clinical Immunology. He is also a member of the World Allergy Organization Asthma Committee. His research also includes several publications on allergy to *Anisakis simplex* 

**Dr. María Concepción López Serrano** received her MD from University Complutense de Madrid in 1968 and her PhD in 1994 from University Autónoma de Madrid. She is a senior consultant allergologist at Department of Allergy, HULP. Her research is mainly devoted to drug and food allergy, particularly to hypersensitivity to *Anisakis simplex*. She has conducted internationally recognized research on Anisakis allergy over the last 20 years. In this regard, Dr. López Serrano has coordinated a Spanish nation-wide multicentric epidemiological study on sensitization to *Anisakis simplex* in the general population (SEAIC, Spanish Society of Allergology and Clinical Immunology 1997). She has co-authored several papers on allergy to this fish parasite.

Dr. Teresa Caballero is a Senior Consultant in Allergology at the Allergy Department of Hospital Universitario La Paz, Universidad Autónoma of Madrid, Spain. She obtained her degree in Medicine at the University of Santander, Spain, in 1986, and her Ph.D. at the Universidad Autónoma de Madrid, Spain, in 1996. She performed her fellowship in Allergy in the Allergy Department in Hospital Universitario La Paz (1987-1990). Afterwards she joined Allergy Department at Hospital Universitario La Paz, where she has been working as a consultant allergist and clinical researcher. She has a broad research interest including hereditary angioedema, quality of life, eosinophilic esophagitis, allergens, food allergy, additive intolerance. She is coordinator of GEAB/SGAB (Spanish Group for the study of Bradykinin induced Angioedema) and is main investigator of an ongoing research project on hereditary angioedema and quality of life funded by FIS (Fondo de Investigación Sanitaria: PI 060843). She has participated as main investigator in several clinical trials in hereditary angioedema. Dr. Caballero has edited and authored numerous publications, including 2 books and over 50 peer-reviewed papers. She is a member of several Spanish and international scientific societies. Her research includes also several publications on allergy to Anisakis simplex.

## B2.2.7 PARTNER 7 HAVSTOVAN - FAROE MARINE RESEARCH INSTITUTE, FAMRI, FAROE ISLANDS

## Description of participant FAROE MARINE RESEARCH INSTITUTE (FAMRI), HAVSTOVAN

The Faroe Marine Research Institute (FAMRI) is a governmental institute with the objective to conduct research on commercially exploited fish stocks, the marine climate and ecosystems in Faroese waters, to communicate the results to the general public, and to provide the Government of the Faroe Islands with advice based on its research. In order to accomplish these goals, FAMRI conducts regular research cruises with the institute's research vessel, Magnus Heinason, focusing on the different components of the ecosystem. Through these activities, FAMRI has collected time series on the physical conditions as well as the different trophic levels of the ecosystem. On a yearly basis, the institute provides the government with stock assessments of demersal fish species in Faroese waters and of straddling stocks, e.g. blue whiting, herring, and mackerel. Because of these investigations the last tventy years FAMRI has succeed in reaching a high level of understanding of the marine ecosystem and the production around the Faroe Islands.

FAMRI is also cooperating with countries worldwide on climate change. This is also because of the geographical position, lying on the frontier between warm and cold oceanic currents, affecting the worlds conveyor belts.

## Attributed Tasks

FAMRI will participate in WP2 and WP9, covering the are around the Faroe Islands and its fishing grounds

#### Relevant experience

The fish industry and the hygienic authorities are requesting educational courses at FAMRI for their staff on the knowledge of relevant fish parasites, concerning human health. This has been done from time to time. At The Faroe Islands the main fish parasites of concern are the following nematodes: *Anisakis simplex, Pseudoterranova decipiens* and *Contracaecum* spp. Guidelines have been developed for the fish producers and exporters in order to meet the demands of the various fish markets in Europe. In these areas FAMRI has a long tradition of collaboration with Norwegian scientists, including scientists at NIFES.

FAMRI cooperates with a number of international research groups and participates in national and international activities and projects. On annual basis, the institute participates in joint cruises on stock assessment and feeding ecology of straddling stocks and (blue whiting, mackerel, herring), plankton and hydrography in EU, Faroese Norwegian, Icelandic and international waters. Simarily, the institute conducts research on demersal stocks and ecosystem research in local waters. FAMRI participates actively in International research bodies (e.g. ICES, NEAFC ets) and in a number of international and national research projects.

#### Staff member profiles

Dr. **Dánjal Petur Højgaard** is an associate researcher to FAMRI (Faroe Marine Research Institute). He is doing various research in fish Parasitology, reaching from small fish (like sticklebacks) to fish for consumption within the fish industry (esp. cod, blue whiting, saithe). He did a his Master's study on the parasites of blue whiting at University of Bergen, 1979-80, under the guidance of prof. em. Bjørn Berland and the late prof. August Brinkmann. In 1998 he defended his PhD on a study of the life-cycle of *Anisakis simplex* and its infection of saithe. This work was done with Dr. Marianne Køie at Marine Lab. Helsingør, Copenhagen University. Since then he has been doing various work in fish Parasitology at FAMRI for the Faroese fish industry, aquaculture industry and the hygienic authorities.

## B2.2.8 PARTNER 8 ISTITUTO SUPERIORE DI SANITA, ISS, ITALY

#### Description of participant

The **Istituto Superiore di Sanità (ISS)** located in Rome is the leading technical and scientific public body of the Italian National Health Service. Its activities include research, control, training and consultation in the interest of public health protection. The Unit of Gastrointestinal and Tissue Parasitic Diseases (UGTPD) of the Department of Infectious, Parasitic and Immunomediated Diseases of the *ISS* performs research, diagnosis, surveillance, and control activities (at both the national and international level) related to both helminthic zoonoses (e.g., trichinellosis, echinococcosis, cysticercosis, anisakiasis, opisthorchiasis and taeniasis) and protozoan zoonoses (e.g., cryptosporidiosis, giardiasis and toxoplasmosis). The UGTPD has been accredited since July 2006, according to the ISO/IEC 17025 international standard. The UGTPD acts as reference laboratory for Trichinellosis for the World Organisation of Animal Health and for the International Commission on Trichinellosis. Since 2006, the UGTPD has been appointed as European Union Reference Laboratory for Parasites (EURLP) by the European Commission. The UGTPD staff is composed of 7 permanent scientists, 6 permanent technicians, 2 non permanent scientists and 2 non permanent technicians.

#### Attributed Tasks

The ISS is the leader of WP6 and will participate in WP 1, 4 and 9

#### Relevant experience

The UGTPD staff deals with foodborne parasitic diseases. In addition to the scientific research, the staff performs parasitological, immunological and molecular diagnoses of

foodborne parasites on different matrices of foodstuff, animal and human origins and epidemiological investigations, for both national and international institutions. Moreover, research and diagnostic activities using experimental animals are carried out according to the EU legislation (CEE 1986 No. 609). The implementation of the quality assurance system, according to ISO/IEC 17025 and ISO 15189, is in progress.

Up to now, no diagnostic tests to detect parasitic infections in foodstuff, animals and humans have been standardized at the European or international level, leading to the need of validation. In this context, most of the Unit staff acquired a long term experience in the specific field of the parasitic disease diagnosis, by implementing the best scientific published information but also by developing new tests and reagents as well as by contributing to the development of international guidelines. These activities, together with the spread of the most up-to-dated methods and techniques to the Italian Health System, are part of the official duties of the ISS.

Staff member profiles

**Dr Edoardo Pozio** is the head of the Unit. He has more than 30 years experience in basic and applied research, in the field of parasitic zoonoses: *Trichinella, Echinococcus, Cryptosporidium, Giardia* and *Leishmania*. He was Director of the Division of Helminthology, Research Director and Director of the Division of Gastro-enteric and Tissue Parasitic Diseases. He headed many national and international research programmes on opportunistic parasitic infections (cryptosporidiosis and toxoplasmosis), *Trichinella* and *Echinococcus*. In 2006, he was appointed as Director of the European Union Reference Laboratory for Parasites. He acts as expert for trichinellosis for the European Commission and EFSA.

**Dr. Maria Angeles Gomez Morales** works in the field of human and animal parasitology. In 1989 she joined the Laboratory of parasitology of the Istituto Superiore di Sanità, where she carried out studies on the humoral and cell-mediated immunity to parasitic infections. She is also involved in diagnostic (preparation and isolation of parasitic antigens and antibodies to be used for diagnostic purposes; standardization of diagnostic procedures) and training activities in animal and human parasitology. At present she is in charge of the Immunology Section at the EURLP.

Dr Gabriella Di Felice works in applied research in the field of immunology, immunopathology and allergology. She has a permanent position at the Istituto Superiore di Sanità since 1985, where she was appointed as Director of the Division of Allergology in 1996 until 2004, in the Laboratory of Immunology. At present she is Research Director in charge of the Allergy Section in the Immune-mediated Diseases Unit of the Department of Infectious, Parasitic and Immunomediated Diseases, Her prevalent interests of research focus on the study of the immune response against natural and recombinant allergens from environmental and food sources. In this field, she headed several national research projects and was involved in European collaboration programmes. More recently, she developed and characterized mouse model of sensitization and anaphylaxis to inhalant and food allergens. These models have been applied to the preclinical evaluation of innovative strategies of tolerance induction and immune modulation of the allergic response by the mucosal administration of hypoallergenic molecules or microbial products including probiotics. Dr Di Felice acts as expert in Italian and European Pharmacopoeia, in the Working Group on Recombinant DNA Plants Modified for Nutritional or Health Benefit and Recombinant DNA Animals, National Committee for the "Codex Alimentarius", and in the EFSA database of external experts.

**Dr. Marco Lalle** joined the Istituto Superiore di Sanità in 2002 as research assistant, and obtained his PhD in Cellular and Molecular Biology in 2003. He carries out studies on the protozoan parasite *Giardia duodenalis*, in particular on molecular genotyping of human and animal isolates, characterization of signal transduction and cell cycle regulation mechanisms.

He is at present part of the Molecular Epidemiology of *Cryptosporidium* and *Giardia* Section

**Dr. Alessandra Ludovisi**, biologist, joined the Laboratory of Parasitology of the Istituto Superiore di Sanità, in 1991, to prepare her thesis on biology of *Trichinella* parasites. Since then, she acquired specific competencies in the use of those instruments (microscopy,

FACS, centrifuge and ultracentrifuge, chemical and biohazard hood, spectrophotometer, etc.) and the performance of methods (cell cultures, proliferation assays, serological assays, etc.) commonly used in an immunological and Parasitological laboratory for diagnosis and research. She participated in studies on the humoral and cell-mediated immunity to parasitic infections, characterization of the immune response to parasites, preparation and isolation of parasitic antigens and antibodies to be used for diagnostic purposes, as well as standardization of diagnostic procedures. She is co-author of several papers on international and national scientific journals and at present is part of the technical staff of the EURLP Immunology Section.

**Mr. Marco Amati** has more than 25 years working experience as laboratory technician at the Istituto Superiore di Sanità. His technical skills and competencies include serological methods (ELISA, Western blot, IFAT), molecular biology techniques (DNA extraction and purification, PCR, PCR-RFLP, multiplex PCR, cloning of nucleic acids, sequencing, Southern blot, electrophoresis in agar or acrylamide gels), collection and purification of parasites from different matrices (tissues, faeces, water, foodstuff), as well as experimental infection, handling, blood collection, necropsy, etc. of laboratory animals. Moreover, he is a very proficient user of Microsoft Windows Office environment (Word, Excel, Outlook Express, Internet Explorer, Power Point, etc.).

**Mr. Tonanzi** joined the Istituto Superiore di Sanità in 1995, and since then he worked as laboratory technician in Parasitology. His technical skills and competencies include molecular biology techniques (DNA extraction and purification, PCR, PCR-RFLP, multiplex PCR, cloning of nucleic acids, sequencing, Southern blot, electrophoresis in agar or acrylamide gels), immunological methods (ELISA, Western blot, IFAT), collection and purification of parasites from different matrices (tissues, faeces, water, foodstuff), as well as experimental infection, handling, blood collection, necropsy, etc. of laboratory animals, including large animals (e.g. calves). Moreover, he is a very proficient user of Microsoft Windows Office environment (Word, Excel, Outlook Express, Internet Explorer, Power Point, etc.).

## B2.2.9 PARTNER 9 UNIVERSITY OF NHA TRANG, IBE, VIETNAM

## Description of participant

**INSTITUTE FOR BIOTECHNOLOGY AND ENVIRONMENT (IBE)** belongs to Nha Trang University (originally University of Fisheries). IBE is composed of the Department of Biotechnology, the Department of Environmental Engineering and the Research and Development Department with the mission of research, training and BOT (Building, Operation and Technological transfer) in the Biotechnology and Environment. IBE is equipped with modern microbiological, molecular and immunological research facilities in order to support education and research. The research programs of IBE mainly focus on not only gene conservation and diseases of aquatic animals caused by parasites, bacteria and virus but also environment impact on marine organisms. In addition, IBE takes in charge of diagnosing the diseases of shrimps and fishes for local farmers as services.

## Attributed Tasks

IBE will participate in WPs 2 and 4, with some effort on coordination and dissemination activities.

## Relevant experience

IBE is a leading research institute in the central region of Vietnam on aquatic animal diseases. IBE has applied modern techniques in the diagnosis of bacteria, viruses and parasites on farmed fish and shrimp. Within the framework of NORAD project financed by Norwegian government, IBE has performed research "Environmental impact of farming activities to the food safety of seafood from blue mussels, babilonia snails in integrated farming systems". IBE also conducts training courses on diagnosis of aquatic animal health

for the veterinary department in the country. Currently, the IBE carried out diagnostic tests on fish and shrimp diseases for farmers

## Staff member profiles

Assoc. **Prof. Ngo Dang Nghia** is expert in food processing. He has long term cooperation with many food factories in Vietnam. Dr. Nghia is principal investigator of the food safety project under NORAD framework from 2003-2008.

Dr. **Dang Thuy Binh** has got her PhD in University of Bergen. Her thesis focuses on monogenic parasites on Vietnamese grouper. She has worked on both morphologic and genetic characters of parasites. Additionally, with background on microbiology and molecular biology, she has done a lot of works regarding on bacteria and virus disease of aquatic animal. She is also deal with teaching She diagnostic methods for aquatic animal disease for Veterinary Departments across the country.

Dr. **Nguyen Van Duy** got his PhD at Institute for Microbiology, Ernst-Moritz-Arndt-University of Greifswald, Germany. He has long-term experience on Molecular Microbiology and Biology as well as transcriptomic and proteomic signatures of *Bacillus subtilis*. His research activities focus now on aquatic animal health in general and applied Microbiology in Aquaculture, Food processing and Environmental Protection in particular.

M.Sc. **Le Phuong Chung** has worked in IBE since 2007. His main research lies in the field of biodiversity and antibiotic activity of actinomycetes. He is also interesting in applying information technology on the researches of biology.

M.Sc. **Nguyen Thi Anh Thu** has an interest in immunology and biotechnology in aquatic organism. During her master thesis in Cell Biology and Immunology group, Wageningen University, she worked on the immunity system of common carp, particularly innate immune reactions against  $\beta$ -glucans. Then she moved to Virology Laboratory in Ghent University for her internship continued working on immune system in a marine organism, in this case, production and characterization of monoclonal antibodies against white spot syndrome virus and host shrimp.

M.Sc. **Van Hong Cam** got her aquaculture master from Ghent University. Her main research is related to monoclonal antibodies production integrated to diseases of marine organisms.

M.Sc. **Nguyen Thi Kim Cuc** is an expert in microbiology and molecular biotechnology in aquatic animals. She has a long time to research in production polyclonal antibodies from chicken eggs against white spot syndrome virus in shrimp.

## B2.2.10 PARTNER 10 ZHEJIANG OCEAN UNIVERSITY OF CHINA, ZOUC, CHINA Description of participant

The university intensifies its discipline construction, carrying forward the integration of production, learning and research so as to promote its scientific research level and social service capacity in an all-round way. At present, it has the provincial "Priority of the Most Important Disciplines" of Fishery Science and Technology, the provincial key discipline of Marine Biology and more than 10 national and provincial innovative platforms. The university has won 1 first prize, 1 second prize and 1 third prize for the National Science and Technology Progress, 6 prizes for National Science Congress, and over 70 provincial and ministerial prizes over the years. Having undertaken a number of the state special projects of modern agriculture, the national "863" projects, the state scientific and technological supporting projects and the state major international science and technology projects, the university has made great contributions to both the regional marine economy and social development. Adhering to taking the school-running path of internationalization and openness, the university is actively carrying out the outside exchange and corporation. It has the International Scientific & Technological Cooperation Base in the marine field, the only one conferred by the State Science and Technology Ministry in China. The university has developed carious forms of running school with such domestic ocean-related universities and institutes as China Ocean University, No.2 Marine Research Institute of the National Bureau of Oceanography, China Fisheries Scientific Academy, etc. as well as

with coastal local governments and departments concerned, and other enterprises. Besides, it has also established the exchange and cooperation relationship with more than 40 universities and scientific research institutes in such countries and regions as the United States, Canada, Japan, Russia and Taiwan, and so on.

## Attributed Tasks

ZOUC will participate in WPs 2 and 4, with some effort on coordination and dissemination activities.

## Relevant experience

Ever since 1980s, Zhejiang Ocean University have been doing research on reproductive regulation and completely artificial breeding of the cuttlefish Sepiella maindroni. The emphasis was placed on a series of researches about fishing, domestication, reproduction, incubations, feeds and disease prevention. Consequently hatchability and survival rate increased enormously and reproductive control and breeding technology broke through. As a result, a perfect technical specification on large-scale seedling culture formed and the artificial breeding cultivation could be performed twice a year successfully. Owing to the multiplication and releasing, the cuttlefish resources recovered. Thus the research of the university became a pioneer in artificial aquaculture of cephalopods in China. It is an initiate research compared with similar technologies nationally and internationally due to the first beginning research, the largest breeding scale and the most papers and patents. During the implementation, 5.25 million zygotes hatched 4.2 million larvae and 140,000 adult Sepiella maindroni survived at last. In addition, 1,221,000 zygotes and 150,000 larvae were released to the sea. At present, two reproduction and culture bases have been built; besides, cultivation and conservation bases are under construction. Moreover, the seedling and cultivation technology have extended to such provinces as Zhejiang, Fujian, Hebei and Hainan, which have obtained remarkable results. As a result of the foundation laid by this research, the resource and quantity of Sepiella maindroni will be repaired gradually in Zhejiang coastal areas are where will be worthy of the reputation of "the Four seafoods zone" again. Finally the Sepiella maindroni mariculture will be carried out both in scale and industrialization similar to large yellow croaker in the east of Fujian.

Staff member profiles

**Dr. Changfeng Chi**, doctor degree in Marine Biology and associate professor at Zhejiang Ocean University of China. She has committed two projects, which are National Natural Science Foundation "Study on the control mechanism of *Sepiella maindroni* neuropeptide on reproductive development" and National Spark Program" Research on key technology development of resource recovery in Sepiella *maindroni*". She has also been involved in 9 other research projects in China regarding to cuttlefish.

Professor Wu Changwen, the Vice president of Zhejiang Ocean University and the Master Tutor, holds a post in various research institutes, such as the chief of Provincial Key Discipline in Marine Biology, the director of the Provincial Marine Aquaculture Engineering Center, the convenor of introducing intellectuals park concerning the marine scientific and technical innovation and so on. Professor Wu has been honoured as the advanced worker and the outstanding expert in science and agriculture technology of Zhejiang province. He has been regarded as the provincial labour paragon and won the national labor medal. Professor Wu has been engaging in teaching and research work about marine biology, ecology and cultivation engineering for a long term. Ever since 15 years ago, he has taken charge of a number of national, provincial and ministerial level research projects, including one state modern agriculture project, five items of The National "863" Plan as well as an array of Provincial scientific research projects. As a result of his excellent research work, a large number of rewards consisting of the state-level and province-level have been available. Main research field: 1.Construction and demonstration on healthy mariculture and industrialization technique system of large yellow croaker Pseudosciaena crocea of high qulity; 2. Studies and demonstration on deep water cages and supporting technology; 3. Research on reproductive regulation and breeding technology in the cuttlefish Sepiella maindroni.

**Prof.Chang Kangmei** graduated in Xiamen Fisheries College majoring in mariculture in Junly 1977. After graduation, he had been working in Marine Fisheries Research Institute of Zhejiang Province until the year 2001. During this period, from September 1987 to

March 1988, he went abroad to study in the Cultivation Fishery Center of Shizuoka Prefecture in Japan. For a long time, he has been devoting himself to the forefront of research and production to resolve critical issues on marine product. He is experienced in artificial breeding such as marine fishes, shrimps, crabs and shellfishes who is also an expert on pond, mudflat, cage and factory aquaculture as well as disease control. Furthermore, he was the first to do research on polyculture of fishes, shrimps and shellfishes as well as mariculture of fresh water fishes in China. At the same time, the high-yield mariculture of Fugu rubripes, *Penaeus vannamei* Boone, and prawns was carried out originally. He had been in charge of series of national, provincial and ministerial level research projects. Since 2006, numerous excellent rewards at the provincial, prefectural and county levels have been obtained. In 2007 a promotion award was granted to him by the People's Government of Zhejiang Province for his contribution to the local mariculture and economic development.

**Lv Zhenming**, Ph.D., associate professor of Zhejiang Ocean University. He was engaged in germplasm resources in marine biology and genetic breeding. He has taken charge of several projects of The International Science and Technology Cooperation Program, National Spark Program, and the Provincial Major Scientific and Technological Cooperation Program. And he took part in several projects as a key member. The project which he participated in the "*Sepiella maindroni* reproductive control and seed breeding technology" got awards of innovation second prize by the State Oceanic Administration and third prize of Provincial Science and Technology Award. In recent years, various types of his papers were published in the journals. In 2007, he was awarded the title of the new century and 151 talents of Zhejiang Province. He had one year research visit for a cooperation project with Norway in Norway University of Life Sciences.

**Dr Jianshe Zhang** received his PhD from Xiamen University in 2008 for research into physiology and ecology of marine animal. Since 2005, he has been involved into various projects regarding to reproductive biology, molecular biology and immunology of Cephalopod. Now, he has a short research visit for a cooperation project of a re-circulating system for mass cultivation of zooplanktonic copepods to be used as alternative, live food in cephalopod culture in ISPRA Istituto Superiore per la Protezione e Ricerca Ambientale of Livorno, Italy, from Dec. 2010 to Nov. 2011.

**Huihui Liu** received her MsD degree from NingBo University in 2004 for work on aquaculture. From 2004 to 2011 she works as a teacher in Zhejiang Ocean University and engages in marine biology research. In 2007 she undertook the Zhejiang Natural Science Fund "The research of Mussels gametes recognition chemicals and their reproductive isolation mechanism". In 2008 She study in Life College of Zhejiang University for a doctor, and she is involved in the research for fish immune.

## B2.2.11 PARTNER 11 CENTRAL LUZON STATE UNIVERSITY, CLSU, PHILIPPINES

Description of participant

The **Central Luzon State University (CLSU)** is one of the well established and comprehensive institutions of higher learning dedicated to the development of research and technologies in agriculture and fisheries. CLSU carries out various fisheries research works including researches on anisakid nematodes. CLSU has been recently carrying out collaborative research studies with various research agencies in Japan (The University of Tokyo) and in the Philippines (National Fisheries Research and Development Institute). NFRDI has currently collaborated with CLSU on the fish stock assessment program in the entire Philippines using anisakid nematodes as possible biological tag for the stock identifications of yellowfin (*Thunnus albacores*) and bigeye tuna (*Thunnus obesus*) in the Philippine-Coral Triangle-Western and Central Pacific Ocean. The strong expertise of CLSU on molecular taxonomy of *Anisakis* and the strong capability of NFRDI in terms of molecular studies and competent manpower having full access to various commercial fishing vessels in the entire Philippines served as a good team in successfully implementing the assigned program of works that are included in the proposal.

#### Attributed Tasks

CLSU will participate in WP2 and 4, with some effort on coordination and dissemination
#### activities.

Relevant experience

Both CLSU and NFRDI have relevant experiences on the anisakid studies since 2006. Both institutions are applying molecular approaches on the implementation of the national tuna stock assessments program of exported tunas around the world where application of parasitological studies is carried out, particularly on anisakid nematodes used as biological tag for their stock assessments in the Philippine-Coral Triangle-Western and Central Pacific Ocean. Both CLSU and NFRDI have been closely collaborating on anisakid studies where both institutions have fully developed molecular biology laboratories that can analyze the taxonomic positions of these zoonotic nematodes. With the current manpower, laboratories, networks and connections in the whole Philippine marine fisheries industry, such anisakid studies in the Philippines is on the right track as a result of increasing awareness in food safety of marine fishes worldwide. The contributions of the Philippine participants in the implementation of this proposal for the Asia Pacific Region would be vitally important in view of human food safety especially on various fisheries products that are being exported to European countries.

#### Staff member profiles

Karl Marx A. Quiazon, Ph.D. had been working on the molecular taxonomy and infection of anisakid nematodes at the Laboratory of Fish Diseases at the University of Tokyo Japan since 2003 obtaining his Ph.D. in 2009. He is working in the field of aquaculture and marine capture fisheries specifically focusing in marine zoonotic nematodes of the anisakid groups in Philippine and Japanese waters. He is currently doing a collaborative research work on the two sibling species of Anisakis simplex sensu lato at the University of Tokyo. They are trying to find out the following: a) epidemiological status of Anisakis infection in marine fishes in Japan waters, b) determine differences on the various allergens present on the two most common Anisakis species in Japan (i.e., A. simplex sensu stricto and A. pegreffii), and c) understand the anisakid's migration behaviour in the body muscles of various host fishes through experimental infection studies. In his home institution in the Philippines (i.e., CLSU), he is continuously doing research works on Anisakis collaboratively doing research work with his co-participant (Dr. Mudjekeewis D. Santos) from NFRDI. Some of his few scientific findings on Anisakis have been published in peerreviewed ISI journals. As a researcher, he is currently spearheading different nationallyfunded projects in the field of fish parasites and diseases, with one project on Anisakis worms which he will be carrying out for 2 years (Nov 2011-Nov 2013) in Japan.

**Mudjekeewis D. Santos,** Ph.D. research accomplishments and current work are centred on applying biotechnology and current molecular methods, in particular genomics or the use of genes and nucleotides (DNA and RNA), to address: 1) problems on the lack of accurate information on aquatic animal taxonomy and population in the natural environment needed for proper management, resource enhancement and monitoring, and 2) problems in aquatic animal health particularly disease monitoring, control and prevention in fish and shellfish species.

#### B2.2.12 PARTNER 12 MAX RUBNER-INSTITUT, MRI, GERMANY

#### Description of participant

**Max Rubner-Institute (MRI)** is the Federal Research Institute of Nutrition and Food with four locations in Germany. Its research focus is health and consumer protection in the nutrition sector. Important fields of research are the determination and nutritional assessment of health relevant food ingredients, the investigation of careful and resource-preserving procedures of processing, the quality assurance of vegetable and animal food as well as the analysis of sociological parameters of nutrition and the improvement of nutrition information.

The Department of Safety and Quality of Milk and Fish (MF) is one out of eight departments of the MRI. The research efforts of the fish quality group of MF, located in Hamburg, shall contribute to the aim of improving the safety and quality of fishery products. Fish and shellfish are studied along the production chain from catch to consumption. Fish meal and fish feed are other important objects of research. Food chemistry, analytical chemistry, biochemistry, molecular biology, physics, microbiology and sensory assessment

are used in an integrated approach to study aspects of seafood safety and quality. The MRI undertakes applied research into various aspects of consumer protection including 24 years of work on parasites of human health significance in fishery products and has a strong publication track record in this field. Many of the research activities are performed in the frame of European projects, with partners from other research institutes, fish processing companies and food control laboratories. The Department of Safety and Quality of Milk and Fish is a core member of WEFTA (West European Fish Technologists' Association). Since 2006 MRI is the seat of the National Reference Laboratory for *Anisakis*.

#### Attributed Tasks

MRI will work in WPs 2 and 6, especially on collaboration with SMEs to develop UV-based methodologies and devices.

#### **Relevant experience**

Many years of experience exist in the determination and identification of nematode larvae like *Anisakis* sp. and *Pseudoterranova decipiens* in fish and fishery products. We have great experience with the digestion procedure and developed the UV-press method, which is today routinely used for epidemiological studies of anisakid nematodes in fish. Earlier work focused on the distribution of nematodes in fish species at capture and a possible migration post mortem under commercial storage conditions. For a long time we concentrated on the prevalence and intensity of *Anisakis* sp. in herring from different stocks important for the supply of the German market. Ongoing research includes other fish species like salmon, mackerel, sardine, saithe, hake, smelt, Grey gurnard etc. We also worked on procedures for killing nematodes larvae during commercial processing and household preparations. As National Reference Laboratory for *Anisakis* we have substantial experience in organizing national ring trials for our official German control laboratories into the quantitative determination of nematode larvae in fish.

#### Staff member profiles

Dr. Horst Karl is a senior scientist in MRI and has over 20 years of experience in epidemiological studies on the occurrence and distribution of nematodes in various commercial important fish species. Based on his investigations minimum processing conditions to inactivate nematode larvae in fishery products were laid down in the former German fish regulation and he participated in the first EU project on nematodes (FAR UP-1-18: Fish parasites of human health significance). He is the leader of the national reference laboratory for Anisakis, the German expert of the Commissions (DG Sanco/ E2) working group on parasites in fishery products and chair of the WEFTA working group on analytical methods. He also works on dioxins and dioxin-like PCBs in fish and fishery products on the quality of new species imported from Asia and tropical countries and were involved in various national projects on quality of seafood from German aquaculture plants. Dr. Ute Ostermeyer received her PhD from Münster University in 1990 for research on a gas chromatographic method for the determination of penicillin residues in animal food. Since 1991 she works in the Federal Research Centre for Fisheries (now MRI) on the determination of mainly positive compounds like vitamins, amino acids and carotenoids in fisherv products. She has been involved in some German research projects concerning the differentiation of wild and conventionally or organically farmed fish. For around four years she cooperates on the National Reference Laboratory for Anisakis.

## B2.2.13 PARTNER 13 UNIVERSITY OF COPENHAGEN, UCPH, DENMARK

#### Description of participant

Professor Kurt Buchmann is in charge of the Laboratory of Aquatic Pathobiology at the Section of Biomedicine, Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark. The laboratory has at present three associate professors, two postdocs, three research assistants, seven Ph.D. students, one technician and three M.Sc. students. The research fields and techniques used comprise parasite diagnostics, histopathology, immunohistochemistry, experimental animal models, PCR, gPCR, ELISA, Western blotting. The group is part of the Biomedicine Section which

is specialized in experimental animal models.

#### Attributed Tasks

UCPH will work on WP2 and 7. Screening Baltic fishes with special emphasis on cod, zoonotic (nematodes, Gadus morhua for potentially parasites trematodes. acantocephalans, cestodes). Samples of these fish species will be taken in ICES subdivision 24 and 25 during winter, spring, summer and autumn. Standard techniques including artificial digestion of tissue will be performed for isolation of worms. Classical and molecular techniques will be applied for diagnosing recovered parasites. Isolated parasites from the field sampling will be tested for their potential zoonotic importance and infectivity of humans by conducting experimental infections of hamsters, mice and rats. Tissue samples will be taken from infected mammalian hosts and subjected to histopathological analysis to describe pathological changes induced by the worm infection. UCPH will also contribute to isolation and characterization of potentially zoonotic parasites in South-East Asiean (Vietnamese) fish products.

#### Relevant experience

The faculty was formerly the Royal Veterinary and Agricultural University founded by the fish Parasitologist Peter Christian Abildgaard in 1773. The faculty is performing education and research within the veterinary and animal health field with special focus on health, food production, food safety, diagnostics, treatment and prophylaxis. Experimental animal model is a focus area in the section of biomedicine.

#### Staff member profiles

**Kurt Buchmann** has been doing fish parasitology research for the last 30 years. The studies include both faunistic field work in wild populations, studies in aquaculture enterprises and experimental work. Studies include European, asian and American geographic areas. During the latest decade molecular and immunological methods have been used with increasing focus in his research efforts. His publication record comprises more than 160 scientific papers within the field.

**Ph.D. student M.Sc. Jakob Skov** has focused on fish parasitology with special emphasis on zoonotic parasites (nematodes, trematodes). He has several scientific publications within the field of human Parasitology. Experienced with experimental infections, PCR, qPCR, ELISA and western blotting.

**Associate professor, M.Sc., Ph.D., Per Walter Kania** is mainly performing molecular studies in fish and parasites. Author of several scientific publications on zoonotic fish parasites and molecular diagnostics. Previous experience in mammalian pathology from the University of Southern Denmark (Department of Pathology).

**Sanaz Mazaheri,** laboratory technician specialized in isolation of parasites, molecular diagnostics, sequencing, histology and immunhistochemistry.

# B2.2.14 PARTNER 14 INSTITUT ZA OCEANOGRAFIJU I RIBARSTVO (INSTITUTE OF CEANOGRAPHYAND FISHERIES), IZOR, CROATIA

#### Description of participant

**IZOR (Institute of Oceanography and Fisheries)** is a scientific institution established for the investigation of the sea (http://www.izor.hr/web/guest/home).

1. Scientific activity conducted encompasses virtually all aspects concerned with sea exploration: physical, chemical, geological, biological and fisheries and aquaculture. In its seventy years of existence, the scientists have published in domestic and peer-reviewed journals, including expedition reports, hydrographic studies, dynamic properties of the marine eco-system, description of flora and fauna, ecological research, fisheries research, advancements in fishing and artificial breeding in aquaculture, as well as man's impact on the sea.

2. Teaching/ training activity has been conducted since 1998 when IZOR developed and established Marine Fisheries and Marine Biology and Ecology Study (http://more.unist.hr/), through the University of Split. IZOR engaging its capacities (laboratories, research

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vessels, classrooms) and human resources to train new cadre of marine biologists and technologists, trained for work in science, aquaculture, fish-processing industry and policy-making bodies. Today, this involves two undergraduate, two graduates and one doctoral course.	
3. Consulting and services: A vast number of services and consulting for private and governmental bodies in frame of environmental and fisheries processes has been offered from IZOR.	
Attributed Tasks IZOR will participate in WPs 2 and 4 on tasks dealing with epidemiological data collection	
and molecular diagnosis	
Relevant experience IZOR has a relevant experience from the execution of the following projects: SeaSearch http://www.sea-search.net/ Your gateway to Oceanographic and Marine Data & Information in Europe. MEDAR/MEDATLAS-II http://ioc.unesco.org/iode/categories.php?category_no=43) Mediterranean archaeology and rescue of oceanographic data programme.	
PELMON <u>http://jadran.izor.hr/pelmon/eng/index.htm</u> Pelagic monitoring-acoustic assessment of distribution and abundance of small pelagic fish	
with monitoring of pelagic ecosystem in the Adriatic Sea. MEDUZA http://jadran.izor.hr/meduza/meduza_g.htm Collaborative international	
research on gelatinous zooplankton of the Adriatic Sea. MAMA http://jadran.izor.hr/mama/hr/index.htm Mediterranean network to Assess and upgrade Monitoring and forecasting Activity in the region.	
ADRIACOSM <u>http://jadran.izor.hr/adricosm/index.htm</u> Adriatic Sea integrated coastal areas and river basins management system pilot project.	
ADRIAMED http://www.faoadriamed.org/Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea.	
SESAME http://www.sesame-ip.eu/Southern European Seas: assessing and modelling ecosystem changes. SUSTAINAQ http://www.sustainaq.net/Sustainable aquaculture production through the use	
of recirculation systems. AQUAMED In development The future of research on aquaculture in the Mediterranean	
Region.	
Staff member profiles <b>Prof. dr. sc. Ivona Mladineo</b> has been working in IZOR (Laboratory of Aquaculture) since 2000 and had her PhD in 2004 in Veterinary Sciences at University of Zagreb, dealing with parasites in marine cage-reared fish. She is working in the field of aquaculture ichthyopathology with special focus on zoonotic diseases transmitted from marine organisms to humans and parasite-host interactions in cage-read system. Since 2001 she has been involved in teaching at the Center for Marine Studies of University of Split, where she coordinates Marine Fisheries course since 2008. She has been actively involved in the EU Framework Program since 2006 and has experience as a partner (SUSTAINAQ, 2006) and work package leader (AQUAMED, 2010). She took part as external EFSA expert in Animal welfare panel in 2008. <b>Dipl. ing. Ivana Lepen</b> Mr. is a PhD student working at IZOR Laboratory of Aquaculture	
since 2009, after obtaining her degree at Faculty of Natural Sciences, University of Split. Her focus is on isolation and identification of immunity-related genes as bioindicators of environmental and biotic stress response of cage-reared tuna using RACE techniques, as well as target genes expression measurement through rearing cycle, inferred by qPCR. She won PhD student award and worked at School of Biological Sciences, University of Aberdeen for 6 months.	
<b>Dipl. ing. Željka Trumbić</b> Mr. is a PhD student working at Center for Marine Studies, University of Split since 2009, after obtaining her degree at the same source. As a PhD student she is full-time engaged within IZOR Laboratory of Aquaculture. Her focus is on physiological response of cage-reared fish to pathogens and currently she is developing tuna microarray for health monitoring at Institute of Aquaculture, Stirling. Her previous experience includes work on bivalves' pathogens, with special focus on <i>Cryptosporidium</i>	

sp. and Giardia sp. in Venus verrucosa and Mytilus galloprovincialis.

### B2.2.15 PARTNER 15 UNIVERSITY OF ABERDEEN, UNIABDN, United Kingdom

#### Description of participant

The **University of Aberdeen (UNIABDN)** is an ambitious, research-driven university with an outstanding history of pioneering discoveries which have changed thinking and practice in medicine, science, arts and humanities over five centuries. Research in the Institute of Biological and Environmental Sciences (IBES) addresses the fundamental biological consequences of environmental change. Within IBES, Oceanlab undertakes a wide range of research, participating in and leading projects in the Atlantic Ocean, Mediterranean Sea, and Indian, Southern and Pacific Oceans. Professor Pierce's "marine and fisheries" research group undertakes a wide range of research related to the life history and ecology of marine species, fisheries and aquaculture, marine food webs, habitat use and ecotoxicology. It has excellent research and training links with other research groups throughout Europe.

#### Attributed Tasks

UOA will lead WP8 (modelling of parasite prevalence, willingness to pay analysis and risk assessment) and participate in WP1 (general project administration), WP2 (data collection on parasites in fished species) and WP9 (dissemination).

#### Relevant experience

Statistical modelling: MFRG has made extensive use of statistical modelling (e.g. GAM, mixed modelling, time-series methods) to investigate relationships between marine species and environmental characteristics, using data on distribution, abundance, life history parameters, diet and contaminant burdens to answer questions about habitat requirements, population dynamics, trophic relationships, bioaccumulation of pollutants and global change.

Willingness to pay.

Risk assessment: Expertise on developing quantitative risk assessment, risk perception, risk mitigation, risk communication, performing exposure assessment, epidemiological methods, integrating lay and technical views of risk.

Market sampling and biology of fished species: The marine and fisheries research group (MFRG) has carried out several projects based on sampling of fish and shellfish from commercial landings and research cruise surveys, as part of studies on various aspects of life history and ecology, including the investigation of parasite burdens.

Specific expertise on parasites.

The group also has experience in GIS and fish stock assessment and links with the UK health service.

#### Staff member profiles

**Professor Graham Pierce** has worked in marine biology and fisheries research for 25 years. A member of the academic staff at University of Aberdeen since 1996, and Marie Curie Chair at the Instituto Español de Oceanografia in Vigo, Spain during 2007-10. He has coordinated several European projects (e.g. CEPHSTOCK on cephalopod biology and fisheries, BIOCET on contaminant bioaccumulation in marine mammals) and is author of approx 190 peer-reviewed scientific papers. He is President of the Cephalopod International Advisory Council and member and former chair of ICES WGCEPH. His research interests are in the life history and ecology of exploited species and top predators.

**Professor I. Theodossiou** has been a participant in the European Low-Wage Employment Research Network (LoWER) and AQCESS (Aquaculture and Coastal, Economic and Social Sustainability; Q5RS-2000-31151). He has coordinated the SOCIOLD (Socio-economic and Occupational Effects on the Health Inequality of the Older

Workforce; QLK6-CT-2002-02292), the EPICURUS (Societal and Economic Effects on Quality of Life and Well being: Preference Identification and Priority Setting in Response to Changes in Labour Market Status; HPSE-CT-2002-00143) project and the HEALTHatWORK (An Inquiry into the Health and Safety at Work; a European Union Perspective; 200716) projects. He has received a number of grants on economic analysis of unemployment. His research interests lie mainly in labour economics, health economics and macroeconomics. He is a Fellow of the Royal Statistical Society and member of the council of the Scottish Economic Society.

**Dr Norval Strachan** has worked on risk assessment and the epidemiology of infectious disease for more than 15 years. He is particularly interested in understanding infectious disease risk and how the findings from this area can be implemented to reduce the disease burden in both human and animal populations. Dr Strachan has published more than 100 papers and has current projects in this area. Dr Strachan has previously worked for Ministry of Agriculture, Fisheries and Food where he looked at methods for detecting parasites in fish and he has also recently served as an eternal expert for EFSA. His contribution to this project will be quantifying the risk of parasites in fish to the European population.

**Dr Lee Hastie** is an aquatic biologist with >20 years research experience in aquaculture systems, fisheries ecology and conservation management. He has worked on giant clam (tridacnid) mariculture in the Indo-Pacific region (1987–1992), invasive species management in Scottish rivers (2006–2008) and the captive rearing of endangered freshwater pearl mussels (margaritiferids) in NW Europe (2000–2006). He has also worked on fish health monitoring in the Scottish aquaculture industry (2004–2006) and recently studied the use of semiochemical repellents to control parasitic sea lice infestations at salmon farms (2010–2011).

**Dr Jennifer M. Smith** graduated from the University of Aberdeen, Scotland in 1999 with an MSc in Marine and Fisheries Science, and was awarded her PhD in Zoology from the same university in May 2011. Her research work focuses on growth and maturation relationships in the squid *Loligo forbesii*, as well as small-scale squid fisheries in UK and European waters. She is currently a postdoctoral fellow at Kasetsart University in Bangkok, Thailand and an honorary researcher at the University of Aberdeen.

**Dr Cristina Pita** is an interdisciplinary researcher working on issues of Environmental Sustainability in the Marine Environment. She has been involved in several EU projects were she focused on the human dimensions of fisheries and environmental management (AQCESS, INCOFISH). She has >10 years experience in questionnaire-based interview surveys, econometric analyses of socio-economic data, and behavioural economics. She is currently a postdoctoral research fellow at the University of Aberdeen.

**Dr Paul Brickle** (Honorary Research Fellow) has over 15 years research experience working on marine fisheries, fish biology and ecology, and parasites of fish and cephalopods. He is currently based at the Falkland Islands Fisheries Department.

**Dr Alain Zuur** (Honorary Research Fellow) is a senior statistician and director of Highland Statistics Ltd. He has written four statistical textbooks and specialises in GLMM and GAMM for spatial and temporal correlated data, and zero inflated data. As a statistical consultant he has contributed to a wide range of projects related to marine biology, oceanography, ecology, fisheries, among others.

**Dr Jianjun Wang** (Honorary Research Fellow, working with the UK Health Protection Agency (HPA)) is a GIS expert who previously worked on fisheries GIS and currently undertakes database management, GIS and analysis of disease prevalence for the HPA.

#### B2.2.16 PARTNER 16 LARPRO ENGINEERING, S.L., LARPRO, Spain

#### Description of participant

**LARPRO** is an independent privately held Spanish Technological Consultancy firm founded in 2009, with a staff of 6. It is specialized in supporting private and public entities in the field of product, process, service innovation, technology transfer and sustainability. LARPRO works with private companies and public entities in different sectors as naval, food, biotechnology and transport. LARPRO can assume different roles in the R&D and innovation projects, taking responsibility for performing the integrated project management, the system requirement definition, taking part of the product development, or performing process modelisation, etc.

#### Attributed Tasks

Participation in WP1 and WP8

#### Relevant experience

LARPRO and it's founders has more than 20 years of experience from setting up, managing and participating R&D projects, at regional, national and international level. The founders of Larpro have setup FP7 proposals and have been project coordinators for collaborative projects (TEFLES). Larpro is managing the participation of several clients in FPVII projects as THROUGHLIFE, and REFRESH, is an active participant of the Europe Innova platform, and is an active member of TII Technology Innovation International network. Has specific experience from project management of large consortiums as the ones mentioned above, and has experience from executing tasks regarding modelling of scenarios, technology applications, economical feasibility analysis, and dissemination actions.

#### Staff member profiles

Christian Larsson, M. Sc. Engineering and M.B.A.

Extensive experience from over 20 years as project manager of complex international R&D projects. Has participated in the following Framework Program projects:

- Has successfully setup and coordinated the FP7 project "TEFLES Technologies for Low Emissions Shipping" (266126)
- Participates in the FP7 project "THROUGHLIFE"
- Participates in the FP7 project "REFRESH"
- Has participated in the project Proposed reduction of car crash injuries through improved smart restraint development technologies (PRISM) FP5 2002-2005
- Has participated in the Network of Excellence on Advanced Passive Safety (APSN) FP6 2004-2005
- IMP<sup>3</sup>rove Innovation Management for Small and Medium Sized Enterprises (SMEs) FP6 2006-2008
- Has managed the following task-relevant projects in the last 5 years:
  - **1.** Project for the International Technology Collaboration: Project for the promotion of SME participation in the 7 th Framework program (2009)
  - 2. Currently finishing PhD on Technology Transfer of Clean Technology.

#### Ana Maria Liñares, degree in Psychology and MBA.

Over 15 years of experience from project management, innovation management and project administration, involving economical issues. Specific FP7 experience from participation in the FP7 project "THROUGHLIFE" and in the FP7 project "REFRESH"

# B2.2.17 PARTNER 17 COOPERATIVA DE ARMADORES DE PESCA DEL PUERTO DE VIGO, ARVI, Spain

Description of participant

This **Vessel Owners' Co-operative of the Port of Vigo (ARVI)** was formally established on the 2<sup>nd</sup> December, 1964. The objective at the time of its establishment was to encourage economic and social improvement of its members, through their combined

efforts, joining forces for a common cause. Nowadays the Vessel Owners' Co-operative and the Associations established within the scope of same have managed to constitute the most important group of shipowners in Spain and one of the most important in the EU allowing us privileged position in the relations with the regional autonomic, national and the EU administrations which are essential in addressing the present and future of the Common Fisheries Policy easing daily negotiations with these administrations, concerning matters of interest to the sector. At present about 260 companies make p the Vessel Owners' Co-operative. The fleet associated comprises of 354 vessels (fish freezing vessels and fresh fish fishing vessels).

Services offered by Co-op to member vessels: - Service for management of fishing licences. - Information and publications service. - Service for accountancy and tax reports. - Service for the management and administration of applications for aids, research, development and innovation. - Service for fresh fish landings. - Service for legal-labour advice. - Service for digital scales. - Service to provide member vessels with reusable plastic boxes. - Service for ships' clearance. - Bunkering and lubricant service. - Service for food/other supplies to the vessels. - Service for fisheries statistics, programs and planning. - Protection and indemnity insurance for member vessel. - Joint service on prevention for ship-owners and shipping companies in the maritime and fishing industries (SPM-COAPRE). - Training activities.

There are nine Fishing Associations and two Producers' Organisations located in the Port of Vigo whose establishment was encouraged by joining fisheries interests which allow for the existence of the Vessel Owners' Co-operative in the aforementioned port.

The Vessel Owners' Co-operative, the Fishing Associations and Producers' Organisations within its scope, participates as a full member in the following national and European institutions: UNACOMAR, CEPESCA, CES, CEP, EUROPECHE, COGECA, EAPO, UE FISHING ADVISORY COMMITTEE.

#### Attributed Tasks

#### ARVI will participate in WP5, WP6, WP8 and WP9.

Relevant experience

The Fishing Associations, the Producers' Organisations and the Vessel Owners' Cooperative make up in 22<sup>nd</sup> June 2007 the Cluster of the Fishing and Fish Producer Sector (CLUPESCA) which represent the most relevant group of fishing fleet industry.

All of them have collaborated for years in fisheries research related issues in Spain, contributing to the development of various research projects run by different research organizations.

Research work has increased since ten years ago and in fact, INNOVAPESCA was created like a unit specialized in research, development and innovation.

- INNOVAPESCA has worked and works in several research fields like:
  - Treatment, management and use of discards and by-products,
  - New fishing gear or methods,
  - Improving the energy efficiency of vessels,
  - Improved fisheries management,
  - Improving the preservation of the catch on board,
  - · Safety on board

In connection with treatment, management and use of discards and by-products ARVI has developed several projects. More projects were focused on aspects of the use of discards and by-products, but we also researched about zoonotic parasites palliation:

We achieved a project entitle "Development of a treatment system of organic waste from cleaning and gutting on board fishing products". A system based on the use of ozone for the treatment of organic waste was developed in the project.

#### Staff member profiles

**José Ramón Fuertes Gamundi:** Degree in Biology by the University of Santiago de Compostela. Managing Director of the Vessel Owners' Co-operative and the different Associations attached thereto. As such, he has acted as main researcher in RTDi projects carried out by the Co-operative. He took part and is currently participating in many bodies and councils representing fishing sector both nationally and internationally.

Edelmiro Ulloa Alonso: Degree in Veterinary by the University Complutense of Madrid.

He holds the position of technical secretary in Co-operative (ARVI). He is responsible for dealing with the general casuistry and designing basic strategies for the associated fleet. He has taken part in several projects carried out by ARVI.

**Jorge Romón Olea**: Graduated in Business Sciences and Social affairs by the University of Santiago de Compostela. He has been heading the department in charge of the management and processing of aids applications, research, development and innovation since 1997. He has coordinated experimental fishing pilot surveys developed by vessels associated with ARVI and has taken part in every RTDI projects carried out by ARVI.

**Bibiana García Soto:** Degree in Marine Sciences. She has Master's degree in Economics and Management of Fisheries and Aquaculture.

She works in ARVI since 2008 in RTD Department. She has taking part in every project carried by ARVI.

**Patricia Docio Pereira**: Degree in Economic and Business Law. She has Master's degree in Fiscal and Taxation Advice.

She works in ARVI since 2007 in Department of Accounting and Taxation. She has taking part in every project carried by ARVI.

#### B2.2.18 PARTNER 18 COMERCIAL HOSPITALARIA GRUPO 3, S.L., CHG3, Spain

#### Description of participant

**Comercial Hospitalaria Grupo 3 (CHG3)**, is a company dedicated to the commercialization of scientific equipment for laboratories, hospitals and industry. Their main research interests are in Molecular Biology, Microbiology, Morphological Sciences, Pathological Anatomy and Forensic Science, Chemical Physics Analysis, Ginecology and special Analysis Techniques.

#### Attributed Tasks

CHG3 will lead the PARASITE Biobank solution (WP3), and it will also participate in the development of a UV-device for parasite detection (WP6)

#### Relevant experience

The company has executed a number of projects regarding detection methods for *Anisakis* spp., including the development of test kit's for the industrial use of the pepsine digestion method, and the mechanical pressing method combined with UV method.

#### Staff member profiles

**Luis Outeiriño Fernández**, have a degree in Biological Sciences by the Universisty of Santiago de Compostela, completed with a Postgraduate Master in Biomedical Research by the University of La Coruña. After finishing his studies he worked in the department of plant genetic resources and genetic improvement of the Mision Biológica de Galicia – CSIC. In 2001, he joined the company Comercial Hospitalaria Grupo 3 SL as responsible of the Microbiology and Molecular Biology Line. From 2007 to date, he is the Sales, Projects and New Markets Manager. Among the functions of this position, is the principal research responsible for the projects that the company have in the area of R + D + I, in collaboration with the IIM-CSIC. Also, has extensive experience in the Organization and Management of various seminars and workshops specialized in Microbiology, Molecular Biology, Life Sciences and Management and Implementation of Biobanks. On this last point, is the responsible and coordinator of the different implementations made in its area of influence.

**Carlos Vello** is the general sales manager. He is in charge of **the** company's sales program, assigning sales territories, setting goals, and establishing training programs for their sales representatives. He has large experience in beta-testing programmes related to proof-of-concepts for new lab products.

**Susana Lema** is at charge of provisions for manufacturing and after-market support. She also attends the standard control procedures for all materials from initial receipt to final product shipment, and document control of quality records. Calibration of testing material is also her responsibility in research and technical projects leaded or shared by CHG3.

Antonio Rodríguez Sousa, degree in Chemical and Scientific Resercher in Comercial

Hospitalaria Grupo 3. Researcher in three different R&D projects for the development of test kit's for *Anisakis* detection for industrial use, over the last three years.

#### B2.2.19 PARTNER 19 TECHNET, TNET, Germany

#### Description of participant

**Technet** is involved in the innovative cores of many projects for which the numerical and experimental mastery of the interaction of form (geometry), material and forces is essential. Technet products are used for realization of widespan structures as the National Stadium in Beijing or the Khan Shatyry Entertainment Centre in Kazakhstan, the development of fish cages for aquaculture or biogas plants. In the analytical sector supervising tools for large scale structures - bridges, ships, historical buildings - and small scale structures - packaging details, food surface or medical X-Ray images - are in the center of activities. For education universities in more in than 15 countries use software systems from technet. Commercial customers are in more than 40 countries. Fields of work:

Development of tools for design, analysis and management of large and small scale structures consisting of multicomponent material. Applications range from structural engineering to analysis of fabrics and biological tissue. Main activities are in the fields

- · Structural engineering and analysis of membranes and widespan structures
- Analysis of surface microstructure and properties of synthetic and natural material
- Laserscanning, Pattern Recognition, Photogrammetry, Image Processing
- Data Analysis and Databases for the handling of multidimensional data

#### Attributed Tasks

#### Technet will participate in WP1, WP6 and WP9

#### Relevant experience

Detection and measurement of position and time dependent surface parameters and their relation to material parameters is a core competence of technet. In the food area this means the classification of local muscle fiber lattice structures and the relation with temperature, pressure and derived quality parameters as freshness, texture or quality variations by freezing-thawing cycles.

Development of a measurement system for registration of tissue changes by freezing for food institutes and food processing. Analysis of X-ray images and CT-scans for medical applications.

#### Staff member profiles

**Michael Kroeger** is physicist and product manager for material analysis systems and has over 20 years experience in image processing and underwater technology for fishery research and fish industry. His area of expertise regards contactless surface inspection by multispectral and 3D image processing and numeric modeling, mainly in the biological, food and medical sector.

**Frank Gielsdorf** is geoinformation scientist and has over 10 years experience in laserscanning and 3D-data analysis. His expertise is calibration of measurement systems, least square methods, regression analysis and statistics.

#### B2.2.20 PARTNER 20 HERMES, S.A, HERMES, Norway

#### Description of participant

**Hermes AS** is a company with one vessel, the freezer trawler M/Tr "Hermes". The vessel fishes all year round and produces approximately 5000 metric tonnes of whitefish and shrimps a year, all caught in the cold and clear waters of the North Sea, the Norwegian Sea, the Barents Sea as well as the fishing grounds around Spitsbergen. The company offers a wide variety of sea frozen products, mainly H/G cod, H/G haddock, H/G saithe (coley), J/Cut or round redfish, J/Cut Greenland halibut as well as industrial prawns.

#### Attributed Tasks

Hermes AS will participate in WP2, task 2.1: Epidemiological data collection. Also it will participate in the beta-testing planned in WP6.

#### Relevant experience

Hermes AS has extensive experience in collecting data for the Norwegian directorate of fisheries during annual surveys of cod, haddock and saithe on the fishing grounds of the Barents sea. Furthermore Hermes AS has developed its own traceability system and made all their traceability data available to the public trough their website. This data also includes QC where parasites are one of many QC parameters recorded. Collaboration with NOFIMA in Tromsø, Norway Hermes AS has participated in several R&D projects mainly in connection to traceability of fish. The latest R&D project Hermes AS is involved in is a project named WITEFISH under the FP7–SME-2011. This project is coordinated by NOFIMA.

#### Staff member profiles

Hermes AS main participants in the project will be general manager Jan Roger Lerbukt and plant/factory manager Hans Ole Tørhaug.

**Jan Roger Lerbukt** has over 15 years' experience as a fisherman, both as plant manager and as captain of a trawler. He now holds the position as general manager and has been the key person in developing Hermes AS's own traceability system. He has been designing and implementing the process for data collection on board as well as the software and the integration between on board systems, and that of the website presenting the data. He therefore has extensive knowledge and experience from catching and processing fish in the factory on board trawlers as well as implementation of new technology.

**Hans Ole Tørhaug** has more than 20 years' experience as fisherman and plant manager both on H/G vessels as "Hermes" as well as factory trawlers making fillets. His most important task is to oversee the production and ensure that the highest quality of finished product is maintained at all times. This includes extensive QC where one of the criteria is the amount of parasites found in the fish produced.

# B2.2.22 PARTNER 21 NEDERLOF'S VISHANDEL B.V., NEDERLOF'S, The Netherlands

#### Description of participant

**Nederlof's Vishandel BV** is a small company dealing in international seafood commerce. The company, counting 12 employees, is located in Stellendam (the Netherlands) and has an annual turnover grows in these years up to about 20 million of  $\in$ . It mainly markets fresh and frozen seafood and is also active in seafood processing and stock.

The main characteristic of the Company is the commitment in the service: its dimension allows staff to care for product quality and to the relationship both with suppliers and customers.

Almost all operations are performed within the Company that is HACCP certified and has an EC number. The assortment that Nederlof's can provide to its customers ranges from North sea species to Argentina fish besides plaice, brills and turbot, cod fillet, pangasius fillet from Vietnam, tuna, swordfish, Nile perch fillet from Africa.

#### Attributed Tasks

NEDERLOF'S will participate in beta-testing trials defined in WP6.

#### **Relevant experience**

PROCESSING AND STOCK

The company has a long experience from dealing in international seafood commerce, processing and stocking fish, as well as assuring that the cold chain and all hygienic requirements are respected based on HACCP protocol.

#### Staff member profiles

Iza Bojanus is head of administration with an experience of more then 20 years.

Erik Slof is Chief Operating Officer of the company with an experience of 20 years in Seafood Industry

### B2.3 Consortium as a whole

#### B2.3.1. Consortium overview and role of the participants

The PARASITE consortium has been setup in order to face the specific challenges and objectives defined in chapter 1.1.1. Partners in the consortium comprise a recognised group of scientific and technical institutions, and research groups involved provide first class complementary expertise in the areas addressed by the proposed project. A group of SMEs, also with complementary skills and deep knowledge on market and societal relevance of the problem addressed by the proposal, will be critical to ensure the capacity of the project to impact the market and the public health problem addressed. All partners have experience in collaborative public-private research activities at national, European and International level.

The involvement of SMEs as active stakeholders will specially focus on methods and tools to be developed in the project to tackle the safety and commercial problems caused by fish and seafood parasites. Such methods will be pre-tested in partner companies and with products being marketed by partner companies. In fact, this proposal puts substantial effort involving partner SMEs in developing beta-testing, concept-proofs and training workshops dealing with detection and mitigation methods for fish zoonotic parasites (and their antigens) and with tools for risk assessment. The group of SMEs involved provides a wide and fairly complete coverage of the relevant species, fishing grounds, processes and markets.

On the other hand, the involvement of Asian partners from three of the major fish providing countries to the EU markets, namely China, Philippines and Vietnam, is a key element to guarantee the impact required by the target Topic Call (KBBE 20.12.2.4-02), given the increasing presence of seafood products from that area in European markets. This collaboration will provide new opportunities to learn more about their practices regarding fish and seafood parasites' risk management. It will also facilitate the implementation of better strategies to ensure the introduction of safety products into our markets. The Consortium includes representatives of the countries with the largest production (excluding Russia), largest trade volumes and value in fishery products and the highest consumption rates of fish within Europe (see Table 2.3.2.1).

#### B2.3.2. Complementarities of participants

The consortium includes all the expertise required for the successful execution of the project. Moreover, the consortium members belong to networks which will significantly strengthen the coverage and capacity of dissemination activities and knowledge transfer. The consortium is well represented in on-going European projects as shown in next section. The participation of the European Reference Laboratory for Parasites will assure together with the other members of the consortium the quality of research performed, the impact on markets and the impact and interaction with policymakers. The integration of feasibility, applicability and cost-affectivity of the technology developed will be assured by involving SMEs from the early stages of each development. The training and dissemination will be assured by the Technology Centre (CETMAR) who will lead the Dissemination Committee.

The multidisciplinary approach of this project has put together researchers and experts with recognised background in areas such as marine biology, food technology, genetics, pathology, engineering, medicine, veterinary, computing, statistics, economy, communication among some others.

One perspective to analyze the consortium is from a triple/tetra -helix perspective. We can conclude that the consortium is well balanced with partners in spheres of innovation.

	PARASIT	E 312068
Innovation sphere	Partner	
Research institutions and academia	CSIC (IIM, MNCN, ICTAN), NIFES, UT-URS,	
	ISS, ANSES, SERMAS, UNIABDN, MRI,	
	UCPH, IZOR, FAMRI, CETMAR, ZOUC, IBE,	
	CLSU,	
Technological development	TNET	
Industrial innovation-integration	CHG3	
Technological consultancy	LARPRO	
End user application	ARVI, HERMES, NEDERLOF'S	
Administration	European Reference Lab for Parasites (ISS)	

Table 2.3.2.1 C	onsortium	overview –	TRIPLE H	IFI IX r	erspective
10010 2.0.2.1 0	011301110111				

The Project therefore integrates representatives of all stakeholder groups. Moreover, the Pan-European dimension and scope given to the project is essential to achieve the objectives of the project and thus, the objectives of the Call. The consortium organised has as one of its strengths the wide geographic scope covered, but it is a key strength also the integration of capacities from so different research interests to tackle the problems caused by seafood parasites from a wide perspective.

## B2.3.3 Subcontracting

Concept	Partner	Amount	Description
RTD	CSIC IIM-E	20.000	Two specialised companies will be subcontracted to undertake: a) one intelligence technological report and periodical alerts of technological vigilance in a six monthly bases, and b) Historic and horizon scanning report of Europe's mass media and social networking perception related to parasite risks in seafood.
MGT	CSIC IIM-E	4.000	Subcontracting of an external auditor (2 audits).
RTD	SERMAS	10.000	Recombinant allergen production will be subcontracted to provide reagents for WP5.
OTH	CETMAR	15.000	Services for the organization of workshop's, video editing, and promotional material for dissemination purposes will be subcontracted in WP9.
RTD	ARVI	40.000	A lab company specialized in DNA will be subcontracted in WP6 to participate in the beta-testing of molecular diagnostic methods by providing Real Time PCR assays in ring trial and proficiency samples.

In all cases, the need for subcontracting rest on the fact that the subcontracted technology requires specialized personnel and equipment not available within the RTD partners. Any subcontract, the costs of which are to be claimed as an eligible cost, will be awarded according to the principles of best value for money (best price-quality ratio), transparency and equal treatment. The method of selection for subcontracting will be established in respect of the applicable public procurement rules as defined in Article II.7 of the Grant Agreement.

## B2.3.4. Third parties

In the PARASITE Consortium one legal entity (Fiorital SL) will participate as third party as a result of a specific "ad-hoc" agreement between the beneficiary and the third party to cooperate in the project. The third party will not work in the project but will contribute to the realization of the project by making available resources to the beneficiary (Nederlofs-partner 21). Fiorital will provide 15,000 square meters as a platform assay for the screening and beta-testing of parasite diagnostic methods. No costs will be claimed by the third party.

## **B2.4 Resources to be committed**

## B2.4.1 Personnel

The required resources related to personnel as well as the allocation of the workforce are shown in Work package description section. Every Work package describes the person-month associated to every partner and task.

[	1 CSIC	1 CSIC-IIM E	1 CSIC-IIM QPM	1 CSIC-ICTAN ES	1 CSIC-MNCN	2 NIFES	3 UT-URS	4 ANSES	5 CETMAR	6 SERMAS	7 FAMRI	8 ISS	9 NTU	10 ZOUC	11 CLSU	12 MRI	13 UCPH	14 IZOR	15 UNIABDN	16 LARPRO	17 ARVI	18 CHG3	19 TNET	20 HERMES	21 NEDERLOF'S	
WP1 ADMINISTRATIVE PROJECT																										
MANAGEMENT	6			0,5	0,5																				0,5	24,5
Task 1.1 Consortium management Task 1.2 IPR management	5	4	0	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	7	0,5	0,5	0,5	0,5	0,5	21,5
WP2: EXPOSURE ASSESSMENT	9,5	9,5	0	0	0	9	8	8	0	0	4	0	15	15	15	14	3	6	6	0	12	0	0	12	12	148,5
Task 2.1 Surveillance of zoonotic parasites of commercial key fish species Task 2.2 Presence of zoonotic parasites	8,5	8,5	0	0	0	9	4	5	0	0	4	0	0	0	0	8	2	2	6	0	4	0	0	12	12	76,5
Task 2.2 Presence of zoonotic parasites in fishery product imports on European key markets: case studies	0,5	0,5	0	0	0	0	4	3	0	0	0	0	0	15	15	0	0	2	0	0	4	0	0	0	0	43,5
Task 2.3 Presence of zoonotic parasites in Vietnamese Pangasius production	0,5	0,5	0	0	0	0	0	0	0	0	0	0	15	0	0	6	1	2	0	0	4	0	0	0	0	28,5
WP3: SAMPLE & DATA MANAGEMENT	14	10,5	0	0	3,5	3	6	0	0	3	0	0	0	0	0	0	0	0	0	0	3	18	0	0	0	47
Task 3.1. Parasite sample management Task 3.2 Epidemiological sample	13,5	10 0,5	0	0	3,5	3	4	0	0	2	0	0	0	0	0	0	0	0	0	0	2	18	0	0	0	42,5 4,5
management WP4: HAZARD IDENTIFICATION	5	1,5	0	0	3,5	0	16	7	0	0	0	0	6	16	16	0	0	9	0	0	0	0	0	0	0	75
Task 4.1 Molecular characterization and genetic structure of anisakids pbased on mtDNA cox2 gene Task 4.2. Genetic identification to the	4,5	1	0	0	3,5	0	16	3	0	0	0	0	2	4	4	0	0	3	0	0	0	0	0	0	0	36,5
species level of anisakid nematodes by MAF	0,5	0,5	0	0	0	0	0	0	0	0	0	0	2	4	4	0	0	0	0	0	0	0	0	0	0	10,5
Task 4.3 Developing of new and inovative nuclear markers	0	0	0	0	0	0	0	3	0	0	0	0	2	4	4	0	0	6	0	0	0	0	0	0	0	19
Task 4.4 Statistical analysis of the genetic data	0	0	0	0	0	0	0	1	0	0	0	0	0	4	4	0	0	0	0	0	0	0	0	0	0	9
WP5: HAZARD CHARACTERIZATION Task 5.1 Antigen characterization for	22,25	1,5	9,75	6	5	0	0	0	0	20	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	46,25
parasites other than Anisakis spp Task 5.2 Antigen exposure (mapping of	6	1	0	0	5	0	0	0	0	5	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	15
allergens) ifor Anisakis spp. in fishery products Task 5.3 Antigen proteomics, including	6,5	0,5	0	6	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11,5
genetic variability Task 5.4 Characterization of the immune	9,75	0	9,75	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14,75
response to the parasite antigens WP6: EVALUATION AND	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
IMPLEMENTATION OF DETECTION METHODS FOR THE INDUSTRY	14	10,5	0	0	3,5	4	5	4	5	0	0	4	0	0	0	4	0	0	0	0	7	6	16	6	6	81
Task 6.1. Evaluation of the mandatory visual inspection scheme Task 6. 2. Technological enhancement of	13,5	10	0	0	3,5	4	0	0	5	0	0	0	0	0	0	2	0	0	0	0	0	0	16	0	0	40,5
the UV-Press method for mass screening of parasites in fishery products Task 6. 3. Implementation of molecular	0,5	0,5	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	6	0	0	0	8,5
methodology based on Real Time-PCR to detect parasites and/or their traces in fishery products.	0	0	0	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Task 6. 4. Development of immune assays to detect parasites and/or their traces in fishery products. Task 6. 5. Validation of the developed	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
and/or implemented methods and evaluation of their performance by Ring Trials.	0	0	0	0	0	0	1	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Task 6. 6. Beta-testing of validated detection methods at industrial level	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	6	6	19
WP7: INTERVENTIONS IN THE FOOD CHAIN TO REDUCE RISKS	33,25	10,75	0	18,5	4	4	1	1	3	2	0	0	0	0	0	0	5	0	0	0	14	0	0	0	0	63,25
Task 7.1 Variation of ithe viablity and infectivity of parasites in fishery products	24,75	10,25	0	10,5	4	4	1	1	3	2	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	40,75
Task 7.2 Interactions between parasites iand bacteria of post harvest fish and their different storage conditions	0,5	0,5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,5
7.3. Tratments for inactivaction of Anisakids in fishery products	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
7.2.2. Development of antigen elimiation or inactivation methiods 7.2.3. Quality of the fish product after	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
selected treatments:	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
7.2.4. Tools to authenticate the technological treatments given to the fishery products	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Task 7.5, Technological enhancement of a device to kill zoonoticanisakids in discarding offalls onboard	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	14
WP8: QUANTITATIVE RISK ANALYSIS Task 8.1. Statistical modelling and	1,5	1,5	0	0	0	3	3	0	4	2	0	0	0	0	0	0	0	0	22	13	0	0	0	0	0	48,5
inference Task 8.2 Evaluate prevalence of human	1,5	1,5	0	0	0	3	3	0	4	2	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	18,5 5
health impacts of parasites in seafood Task 8.3 Quantitative risk assessment	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	5
Task 8.4. Analysis of willingness to pay Task 8.5 Cost/Benefit analysis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	5
WP9: INNOVATION, COMMUNICATION AND DISSEMNINATION	8,6	3,8	0,6	3,2	1	3	3	0,6	23	3	0,6	2	0,6	0,6	0,6	0,6	0,6	0,6	3	1,5	1,5	1,5	1,5	0,6	0,6	57,6
Task 9.1. Catalogue/Portfolio Task 9.2. Technology transfer supporting	6,6	1,8	0,6	3,2	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9,6
1035 7.2. IECHNOLOGY transfer supporting		0	0	0	0	3	3	0,6	6	3	0,6	2	0,6	0,6	0,6	0,6	0,6	0,6	3	1,5	1,5	1,5	1,5	0,6	0,6	32
workshops.	0		0	0	0																					
workshops. Task 9.3. Roadmapping future prospects. Task 9.4 Development of communication	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
workshops. Task 9.3. Roadmapping future prospects. Task 9.4 Development of communication	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
workshops. Task 9.3. Roadmapping future prospects.	0	0																								

#### B2.4.2 Other direct costs

Travel costs:

- Technical meetings, and travel expenses for testing Part of (RTD cost)
- Participation in project meetings for the follow up of the project. This means steering committee meetings (every 6 months), consortium meetings (every year), etc. (Part of Management cost)
- Meetings and workshop's for dissemination, end user visits etc. (Part of Other costs)

Concept	Total	CSIC	NIFES	UT- URS	ANSES	CETMAR	SERMAS	FAMRI	ISS	IBE	ZOUC	CLSU
RTD Travel	85.000	13.000	5.000	5.000	5.000	5.000	3.000	3.000	3.000	4.000	4.000	4.000
MGT Travel	111.000	17.000	7.000	7.000	4.000	4.000	6.000	6.000	6.000	0	0	0
OTH Travel	37.000	5.000	3.000	3.000	0	3.000	0	0	0	6.000	6.000	6.000

Concept	MRI	UCPH	IZOR	UNIABDN	LARPRO	ARVI	CHG3	TNET	HERMES	NEDERLOFS
RTD Travel	3.000	1.500	1.500	5.000	5.000	3.000	3.000	3.000	3.000	3.000
MGT Travel	6.000	3.000	3.000	5.000	7.000	4.000	4.000	4.000	4.000	4.000
OTH Travel	0	0	0	2.000	3.000	2.000	2.000	2.000	2.000	2.000

#### Consumables and equipment:

The partners will use routine consumable (fish samples, lab tests, reagents,...) and dissemination materials.

Concept	Partner	Amount	Description
RTD	CSIC	58.500	Fish samples, lab test consumables, reagents,
RTD	CSIC IIM-E	8.000	Fish samples, lab test consumables, reagents,
RTD	CSIC IIM-	7.500	Fish samples, lab test consumables, reagents,
	QPM		
RTD	CSIC ICTAN	25.000	Fish samples, lab test consumables, reagents,
RTD	CSIC MNCN	18000	Fish samples, lab test consumables, reagents,
RTD	NIFES	12.000	Fish samples, lab test consumables, reagents,
RTD	UT-URS	64.500	Fish samples, lab test consumables, reagents,
RTD	ANSES	65.000	Fish samples, lab test consumables, reagents,
OTH	CETMAR	6.000	Dissemination consumables
RTD	SERMAS	40.000	Fish samples, lab test consumables, reagents,
RTD	FAMRI	15.000	Fish samples, lab test consumables, reagents,
RTD	ISS	80.000	Fish samples, lab test consumables, reagents,
RTD	IBE	9.000	Fish samples, lab test consumables, reagents,
RTD	ZOUC	18.000	Fish samples, lab test consumables, reagents,
RTD	CLSU	18.000	Fish samples, lab test consumables, reagents,
RTD	MRI	28.000	Fish samples, lab test consumables, reagents,
RTD	UCPH	4.500	Fish samples, lab test consumables, reagents,
RTD	IZOR	13.500	Fish samples, lab test consumables, reagents,
RTD	UNIABDN	8.400	Fish samples, lab test consumables, reagents,
MGT	LARPRO	32.000	Project management software
RTD	ARVI	3.600	Fish samples
RTD	CHG3	59.000	Fish samples, lab test consumables, reagents,
RTD	TNET	3.100	Fish samples, lab test consumables, reagents,

Additionally, durable equipment will be needed as follow:

Concept	Partner	Amount	Description			
RTD	CSIC IIM-E	45.000	RT-PCR equipment for participation in ring trial; ultrafreezer for			
			samples backup in the Biobank; laboratory test material			
RTD	NIFES	8.000	Equipment for further automating the pressing method; parasite			
			and tissue storage equipment			
RTD	UT-URS	20.000	Equipment for pressing method; -80°C ultrafreezer and -20°C			
			freezer for samples; power supply for DNA analysis			
RTD	ANSES	4.000	UV-Press equipment for ring trial			
RTD	MRI	16.000	Equipment for further automating the pressing method			
RTD	IZOR	15.300	Nanodrop spectrophotometer			
RTD	UNIABDN	2.000	Laptop computer			
MGT	LARPRO	32.000	Software for project management of consortia			
RTD	ARVI	23.400	Equipment for installation in fishing vessel			
RTD	CHG3	126.000	Bio-E-Bank software including external hosting for global support			
			of the solution; 2-D labelling systems and associated software			
RTD	TNET	18.200	Equipment for sample preparation (mech component), camera			
			system, optical lens, LED illumination, computer, image			
			processing device.			

# B2.4.3 Detailed budget Partner 1 CSIC

The required resources for partner 1 CSIC are the following:

	CSIC	CSIC-IIM E	CSIC-IIM QPM	CSIC-ICTAN	CSIC-MNCN
RTD ACTIVITIES					
Personell	311.650	123.525	34.125	85.750	68.250
Other direct costs	118.000	60.000	9.000	28.000	21.000
Indirect costs	468.417	226.051	62.449	82.320	97.598
Subcontracting	20.000	20.000	0	0	0
Total	918.067	429.576	105.574	196.070	186.848
DEMONSTRATION ACTIVITIES					
Personell	0	0	0	0	0
Other direct costs	0	0	0	0	0
Indirect costs	0	0	0	0	0
Subcontracting	0	0	0	0	0
Total	0	0	0	0	0
MANAGEMENT					
Personell	17.000	13.500	0	1.750	1.750
Other direct costs	17.000	9.000	0	4.000	4.000
Indirect costs	28.888	24.705	0	1.680	2.503
Subcontracting	4.000	4.000	0	0	0
<u>Total</u>	66.888	51.205	0	7.430	8.253
OTHER ACTIVITIES					
Personell	27.060	10.260	2.100	11.200	3.500
Other direct costs	5.000	3.000	0	2.000	0
Indirect costs	38.376	18.776	3.843	10.752	5.005
Subcontracting	0	0	0	0	0
<u>Total</u>	70.436	32.036	5.943	23.952	8.505
TOTAL BUDGET	1.055.390	512.817	111.517	227.452	203.605
REQUESTED EC CONTRIBUTION	825.874	405.423	85.123	178.435	155.155

# **B3. Potential Impact**

## B 3.1 Strategic impact

The PARASITE project, offering a **multidisciplinary approach** to address the risk assessment related to the presence of zoonotic parasites in seafood of European markets, will have various impacts at different levels and scales. The integration of all the scientific data achieved in the project will have strategic impact on three fundamental issues:

- Scientific support to European Food Safety Policy.
- Improving competitiveness of fish and seafood producers and markets.
- Consumers and society from a global perspective: impact on market perception and on Public Health.

## B3.1.1 Scientific Support to European Food Safety Policy

The project will contribute to food safety policy by addressing the research needs identified in the EFSA scientific opinion on risk assessment of parasites in fishery products.

EFSA recommends that research should be improved on:

• The infectivity as well as inactivation of parasites in fishery products in relation to treatments, host fish species, and effects of passage through different hosts.

The PARASITE project deals with this aspect mainly in WP7, Task 7.1 (infectivity and viability of parasite) and Task 7.2 (interaction bacteria-parasite). In Tasks 7.3., 7.4 and 7.5 optimal treatments to inactivate the parasite and its antigens, both in products and in fish offal and discards. Results of this WP will mainly materialize through a set of five deliverables:

D.7.1. to D.7.4 will mainly provide scientific bases to bridge the gap identified by EFSA. The efforts to spread progress on scientific knowledge (see WP9) will be aimed to guarantee the use of such results by orienting the policymakers and providing new evidences to the Scientific Community. D.7.5. 'Prototype for management on board of parasite contaminants in residues', and D.7.6. 'Guidelines for parasite risk management in the food chain' are the main outputs in WP7, expected to impact by effectively reducing the risks related to fish parasites in the production chain. Treatment means and strategies will be to offered/recommended to the European industry for being able to guarantee their products are innocuous for consumers, and demonstrating their commitment in responsible and sustainable fishing practices.

Trials in real-life conditions will be positive for the industrial involvement and thus for a former spread of the market implementation of results. Dissemination activities targeting the industry regarding WP7 will strongly concentrate in these two deliverables.

• The effects of different farming practices on the prevalence of anisakids in aquaculture if products are to be consumed raw or almost raw.

In European aquaculture systems, despite of off-shore facilities for on-growing of Tuna in the Mediterranean Sea, and experimental culture of octopus in the NW of Spain, feeding systems are rarely based on fish offal and/or discards, so the risk for European aquaculture products to prompt allergic reactions in fish consumers due to parasites is not too high.

However, in fish from exporting Asian countries we can find viable parasites (anisakids and other helminths) with allergenic capacity as it has been described in the State of the Art section of this document. It is important to know more about the farming practices in these countries, the prevalence and distribution of parasite hazards.

The WP2 expected result is mainly a complete dataset on epidemiology of anisakids in main EU fishing grounds that will feed WP2 and WP8. In WP2 will be the base to build a geo-referenced Information tool on zoonotic parasites in fish stocks, useful for fisheries and food safety authorities to get a clear overview on the situation and to build on it monitoring programmes to know more on the evolution of the problem. It will also be useful for the industry to implement self-control systems.

In WP3, the PARASITE Biobank is a key tool for the project implementation and an output of interest for research bodies and food safety authorities as far as its information and biological resources with certified traceability guaranties will be at their disposal. Besides it is expected to keep on supporting bio-sampling storage and management once the PARASITE project has finished.

WP4 will provide key tools to perform risk assessment (WP8) as far as it is known that not all the *Anisakis* species have the same zoonotic relevance to humans. Population genetics' estimations, in task 4.5., will be also a factor for risk analysis and management.

Samples from imported species will also be analysed in WP2 and 4 aiming to get a realistic overview on the presence of anisakids in relevant fish imports arriving in EU markets.

Information on anatomic allocation of relevant for health parasites will also be studied, and strategies on fish handling to avoid commercial looses due to parasite infestations will also be identified and disseminated to relevant agents. Industry and control authorities will have the opportunity use simple and fast/mass detection methods and to integrate them in prevention, self inspection and control plans. Training workshops and dissemination activities will be accomplished as main paths to reach the relevant public and impact on it with project results. In WP6, the detection methods and viability assessment techniques will be integrated in new devices and tools will also be developed and tried by companies under beta-testing experimental designs. This beta testing work will effectively allow assessing viability and validate the industrial usability of such results.

In WP8 the data obtained in WP2 and from WP4 will constitute an input to develop statistical models that predict the incidence, distribution, variability and consequences of parasites in different products and markets, including the consumer perspective and its willingness to pay for *Anisakis*-free products. This will mainly allow the industry to take management decisions (self-control, labelling, etc.) on how to allocate its products and what to emphasise in marketing campaigns. Policy-makers will be supplied with key information to build on feasible scenarios and take decisions to guarantee consumers' interests and safety without detriment of industrial competitiveness and employment.

Besides the above stated, EFSA also recommends that co-ordinated studies are carried out to improve surveillance and diagnostic awareness of allergic reactions to parasites in fishery products. This part of the work, more directly related to human health impact, is tackled in WP5. The expected result is a clear characterization of allergenic capacity of anisakid species other than *Anisakis simplex* and the immune response to anisakids antigens. This information will be of key relevance to determine the consequences of anisakids to public health and thus, to implement measures that should be efficient in reducing the risks to population. The project is aiming to yield the basic information and tools to facilitate the implementation of strategies that can mitigate the problem without causing a dramatic decrease in fish consumption that would, on the other hand, have a negative impact in public health.

Permanent dialogue with food safety authorities will be the main path to reach the above stated EU food safety policy. This will be achieved by:

The EU Reference Laboratory for Parasites (ISS) is one of the PARASITE partners it will not only constitute a direct channel to the transfer of information to EU authorities but also to receive a direct feedback from them.

Representatives from national and EU authorities will be invited to integrate the project's Dissemination and Exploitation Committee and they will always be invited to participate in dissemination activities planned in WP9 such as training workshops, dissemination seminars and symposium, discussion panels, etc.

Relevant health and food safety authorities will be invited to have access and try some of the tools developed in the project with the aim to provide an overview of the situation (geo-referenced database), design scenarios (risk management models) and manage information and resources (Biobank).

Improving competitiveness of Fish and seafood producers and markets.

In the above explanation about impact on policy it can also be deduced the relevance and expected impact of the PARASITE project to the industry. However one very relevant aspect that is not so evident from the above explained relates to the way the project has been designed to broadly branch the fish and seafood value chain, providing outcomes that will be applicable from the net to the plate.

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According to FAO and Eurostat data, EU-27<sup>1</sup> is the fourth largest producer of fisheries and aquaculture I the world. If we add the production of Norway to that of the EU, then Europe is the second largest producer of fish and seafood products. Spain, France, UK, Denmark, Italy and the Netherlands (all countries represented in the consortium) rank among the first EU producers in this order.

Employment in the catching sector, according to the same source is over 141.000 and according to FEAP<sup>2</sup> in aquaculture in Europe represents some additional 60.000 to 70.000 people. If we add to this the processing sector and related industry, we reach the estimations of more than 340.000 people made by FAO and mentioned in section B.1. in this project.

 Processing industry in Europe is made of more than 4.000 companies, most of them small and medium sized business, many of them with 20 employees at most. Most relevant fishing countries are also those with a most prominent processing sector. This activity multiplies the value of the primary production more than three times but its relevance in global terms has to do much with the social repercussion fisheries related activities have in many coastal rural areas in Europe.

Europe is not only a major fish producer but a world-class fish consumer. While the worldwide average consumption of fish per capita and year is 16.4 Kg., for the EU it is 22.3 with some countries ranking far high from the average, as Portugal (55.6 Kg); Spain (41.2Kg); France (35.3Kg).

International trade has gained more and more importance in the last decades and the global
picture hereunder is also relevant not only for the economic relevance of the data provided, but
also for the countries that namely appear in the picture; many of them are represented within the
PARASITE consortium. Along with Japan and the USA, European Union is the third main fish
and seafood importer in the world.

		Imp	orts	Expo	orts
Volume in tonnes			$\bigcirc$		Ð
Value in thousands of EUR	Pelagic fish	1 127 528	2 615 663	1 010 885	1 135 077
	Salmonids	618 616	2 286 766	50 635	238 922
Tuna, sardine, mackerel, herring, anchow, etc.	Other fish	1 928 306	6 063 445	353 847	899 278
Salmon, trout,	Crustaceans and molluscs	1 313 202	5 089 055	164 145	493 527
Cod, hake, pollock, haddock, panga, sole, halibut,	Non-food products	669 070	485 034	194 950	167 272
sea bream, etc. Shrimo, spiny lobster, scallon, mussels, cuttlefish	Total EU-27	5 656 721	16 539 963	1 77 4 462	2 934 076
Shrimp, spiny lobster, scallop, mussels, cuttlefish, squid, etc.		0000721	10 007 700	1774402	2754070
Products not intended for human consumption, fish meal, decorative fish.					

The PARASITE project is made of a consortium of partners coming from Spain, France, Germany, Italy, Denmark, Norway, UK and Netherlands. the lt is not surprising that the call topic puts special emphasis on the interest to consider imports from Asian countries giving the increasing trend of products from these countries entering EU markets. Partners from China, Vietnam and Philippines institutions enlarge the list of countries represented by the consortium and this, together with the Workplan designed it will facilitate the exchange of

experiences and know how with these countries with the aim to guarantee the products entering EU markets meet the same high quality, control and safety standards.

To add more information on the relevance of imports for the problem addressed (presence of parasites susceptible to cause allergies and commercial looses), hereunder there is data on how much imports of fresh or low processed products represent to the total:



Source: Facts and Figures on Common Fisheries Policy 2010.



Source: Facts and Figures on Common Fisheries Policy 2010.

Very recently, in September 2011, the EU Fish Processors and Traders' Association released the report "Finfish Study 2011". In this study the industry assumes that imports are absolutely necessary to satisfy the growing demand and to produce value added seafood in Europe. The study recognises that the introduction of foreign species in EU markets has been key to respond to consumer expectations and needs.

The EU authorities and the industry itself invest heavily to make sure that all the fishery products they handle, whatever their origin, comply with the highest standards of food safety, nutritional value and consumer appeal. However efforts in safety and traceability must continue to make EU standards more widely adopted. Transferring knowledge to major supplying countries and on the other hand knowing more on their lacks to comply with the EU safety, ethical and environmental standards will help improving the mechanisms to introduce their products with total guarantees and in fair competitive conditions.

Apart from the possibilities to exchange experience with some of the most relevant Asian fish providers, the strategy designed within the PARASITE project has included fish and seafood imports

as part of the whole chain that will be impacted by the outcomes expected from the accomplishment of the project. In general terms, having this perspective in mind, PARASITE project will contribute to increase the confidence of consumers in seafood products and thus to enlarge the market and increase the industry competitiveness by facing one of the major challenges for supply (safety guarantees).

Project results are expected to provide technologies and knowledge upgrade applicable in most of the seafood chain elements. Results from WPs3 and 6 are relevant for self HACCP systems applicable to production, processing and imports: Geo-referenced epidemiological database (resulting from WP3) and detection methods adapted for industrial use (resulting from WP6). Results from WP7, mainly guidelines and treatment methods to kill and or inactivate parasites, specially the device to treat fish offal and discards will again be applicable in all the chain, from catch to consumers. Companies from different value chain segments and industrial associations at European scope will be the target for training and dissemination activities to be accomplished. Moreover SMEs in the project will, with their own experience, serve as reference point for dissemination among the industry.

#### Consumers and society at a global perspective. Impact on market perception and on Public Health.

The development of food allergies is an increasingly common concern among consumers worldwide. Besides the considerable reduction in product quality due to anisakid nematode larvae in fish, the parasites are zoonotically important. In Europe, human infection with live *Anisakis* spp. larvae, a condition referred to as anisakiasis, is most frequently reported from certain regions of Spain and Italy where raw or lightly salted or marinated fish is part of the regular diet.

Alerts reported in the last years have boosted the public authorities (European and at MS) to regulate on food safety aspects including a range of preventive control measures to be applied by the industry and food providers in order to minimize the health risk to consumers from the possible presence of parasites in fishery products.

The special focus the PARASITE project makes in developing solutions that can be industrially implemented and in providing tools to support food safety authorities' decisions are two of the key elements to achieve a reduction of risk exposure, given that prevention is the most effective approach to tackle this hazard. However the project will also yield new insights on the genetic and proteomic variability of zoonotic anisakids linked to infectivity patterns, antigen characterization and clinical findings. Progress on knowledge about these aspects will allow the health bodies to improve diagnostics, treatment and to be more effective in designing prevention programmes. In WP7, work related to the determination and assessment of viability and infectivity of anisakids in commercial products, will also yield conclusions with direct implications in public health. One of the expected outcomes for this WP is a guideline document that will offer consumers and fish suppliers, alternatives to treat products with safety guarantees regarding the zoonotic risk addressed and without detriment of products' quality attributes. Risk assessment focus on dose-response, cost-effectiveness and consumer behaviour analysis addressed in WP8 will provide clear conclusions on most efficient measures to manage different possible scenarios.

The proposed project offers to provide major contributions for minimising the risk of parasite contamination of fishery products reaching the European consumer. In a longer perspective, the project may significantly contribute to retain or even increase the average per capita fish consumption rate in Europe through strengthening consumer confidence in marine fish from European fishing grounds and markets as safe and healthy food.

In WP9 it has been defined a set of actions that will enhance the achievement of the above described expected impact. Regarding public health implications paths to guarantee impact will mainly consist in spreading knowledge achieved among the medical societies and health authorities by presenting to them the project outcomes in their regular meetings and congresses, and also through recognised scientific publications. As far as some of the results are expected to have clear implications to the fishing business, alternatives on treatments will be widely disseminated to companies, associations

and other platforms and the experience of partner SMEs in this sense will reinforce the capacity to reach the target audience and help them voluntary adopt some of the measures identified.

Industrial partners will also become key agents in transferring the relevant information and messages about the project outcomes to the consumers, they will analyse opportunities for labelling but also use the means the project puts at their disposal to interact with their customers and market the effort done.

By developing consumer confidence in all seafood products the market has been able to grow significantly despite the challenges of supply, new and positively oriented efforts in this sense are expected to yield in new opportunities for increasing seafood industry competitiveness. EU sourced materials of both wild capture and aquaculture origins have considerable opportunity to contribute to the future of the European markets and the industry will welcome the development of EU fisheries so they can increase their share. This does not necessarily replace products that are currently imported as the scope for expansion will require additional resources.

The effects on consumers' perception will be addressed in the project first to analyse most probable scenarios about its willingness to pay for anisakids-free products, being this, a key element in risk analysis (WP7) and second by working on dissemination and communication activities with consumers as targets of many relevant and practical information coming out from the project.

# B3.2 Plan for the use and dissemination of foreground-Dissemination and/or exploitation of project results, and management of intellectual property

#### B3.2.1 Dissemination strategy

WP9 will be the source to provide all the other WPs with information, materials and means for supporting an efficient implementation of the actions foreseen in the Knowledge Transfer, Dissemination and Communication Plan. A wide range of dissemination and training supporting materials will be provided: leaflets, posters, handbooks, presentations, web facilities ... The, audiovisual means will allow to replicate the planned training activities in different locations without relevant additional cost, so that a great number of final users can be reached.

The targeted dissemination actions and spreading of information about parasites in general and their mitigation treatments are needed in order not to raise unwarranted consumer alarms or harm seafood producers, and not to risk a reduced consumption of seafood. This will be achieved by following in all the dissemination means the recommendations of the European Food Information Council (workshop "Quo vadis in food risk communication", 2004). Special care will be taken in identifying and characterising the information needs and preferences of target public, in crafting adequately the messages to reach them, in generating trust and becoming, as a group, a reference source of information about *Anisakis* related risks, etc.

Any dissemination activities and publications in the project, including the project website, will (i) specify that the project has received Community research funding and (ii) display the European emblem. When displayed in association with a logo, the European emblem will be given appropriate prominence. All publications shall include the following statement (from GA art. II.30.4): "The research leading to these results has received funding from the European Community's Seventh Framework Programme under Grant Agreement No. KBBE-x (Project ACRONYM)".

In support to the communication activities of the Commission services, and in addition to a presentation leaflet that it may initiate, the consortium may be requested to provide the Commission with a 2 pages information sheet (double sided A4) which will be drafted in a standard format communicated by the Commission. Upon request, the consortium will also provide an updated version of this information sheet. The Commission services may also request one illustration (picture, schema or drawing) to illustrate such communication material.

Following the proposed table reflects how PARASITE partners identify the structure of the target public for its dissemination and transfer activities, and provides clear specific examples of who would integrate such target groups:

Target Public	Definition	Examples (non exhaustive list)
Scientific community	Researchers, research groups and/or research institutes dealing with fisheries, aquaculture, seafood processing, food safety and health issues. This group will be mainly addressed by means of presenting project results in relevant conferences and publication of papers in key journals	Discussion forums: WEFTA, ICES, NAFO, EAS, EAACI, OIE, European Association of Fish Pathologists, SeafoodPlus Research Platform. Events:International Symposium on Fish Parasites (IX ISFP, Valencia, Spain), International Symposium in Marine Sciences, International Symposium Strategies for management of Parasitized Seafood Products, Food Allergy and Anaphylaxis Meeting Scientific Journals: Journal of Fish Diseases, Parasitol Res., Vet. Parasitol.,Fish Pathology, Fisheries Research, Journal of Fish Biology, ICES Journal of Marine Science, Trends in Food Science and Technology, Food Science and Technology; European Food Research and Technology, Food control, Journal of Food Protection, Int. J. Food Microb., etc. http://www.openaire.eu/en/home Others: ERA-NETs (SeasEra,), JPI Healthy and Productive Seas and Oceans and other relevant EC- funded initiatives; FederCoopesca, FederPesca, LegaCoop,
Health and Food Safety Authorities	Public decision and policy-making bodies in charge of taking care and issuing regulations on health, food processing, animal feed, international trade, labelling, etc., both at national and European level. Some members of this group will be invited to participate as external advisors (Exploitation and Dissemination Committee).	Directorate Generale SANCO, EFSA, National Food Safety Authorities, National Health Authorities, National Reference Labs, Inspection Bodies, DG TRADE, National Ministries for food and international trade, FAO, WHO, WTO ERA-NETs (SeasEra,), JPI Healthy and Productive Seas and Oceans and other relevant EC funded initiatives

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Fisheries Authorities	Public decision and policy-making bodies in charge of fisheries and aquaculture management an regulation, both at national and European level Some members of this group will be invited to participate as external advisors (Exploitation and Dissemination Committee)	DG MARE, National and Regional Ministries for fisheries and aquaculture ERA-NETs (SeasEra,), JPI Healthy and Productive Seas and Oceans and other relevant EC funded initiatives
Industry & Marketing	Producers and marketers along the seafood value chain, covering fishing, aquaculture, food processing, distribution and international trading companies. They will be mainly addressed through sectoral organisations and by means of industry- targeted seminars, technical publications and relevant trade fairs. Some members of this group will be invited to participate as external advisors (Exploitation and Dissemination Committee)	Technology Platforms: EATIP, EFTP, Food for Life,ETPGAH, National and Regional Technology PlatformsSectoral Organisations and Associations:Europêche,FEAP, FoodDrinkEurope, EU Fish Processors andTraders Association, National Organisations of producers.Technical publications:Seafood International, WorldFishing&Aquaculture, and other similar publications atnational levelTrade fairs:European Seafood Exposition, NorFishing,Conxemar, Alimentaria and other trade fairs related toseafoodEvents:International Symposium Strategies formanagement of Parasitized Seafood Products,
Consumers	Stakeholders demanding and purchasing seafood products	BEUC (European Consumers' Organisation): Food, Health, Environment and Safety Department -> this will be the elected delegate for contacting national organisations if necessary, as most of them are members of its board (http://www.beuc.org/Content/Default.asp?PageID=1527)
Civil Society	General public concerned about health issues and potentially exposed to the risk of seafood-borne parasitic diseases	Dissemination through general media such as mass media, open forums, websites, etc.

The following table "portfolio of transferable results" summarises this information being more precise in identifying the elements into which results will materialise (Deliverables) and in relating to them the kind of dissemination and technology transfer support actions that will be performed for each case and for each kind of target public.

	Portfolio of transferrable/spreadable results           Result/         Available         Partners in         Dissem.						Input for	
	Result/					Туре	e of activity	Material/
	Related Deliverable	month	charge	level*	public	Training	Dissemination	Format
	Technology-based Biobank for zoonotic parasites in fishery products aligned with a open public access plan post p regulatory bodies, researchers and industry in Europe. The Exploitation plan will include that partner 18 (the benefic Biobank) will cluster this technical solution with other successful KBBE proposals within the FP7 (or even previous	Scientific		Presentations in public events and Symposium				
	D3.1. Database of storing parasites, DNA samples, specific and characterized antigens and parasite specific immune sera	36	CSIC IIM	PU	Community Health and	Use of Biobank	on Strategies for Fish and Seafood Parasites' management	Handbooks, presentations, posters
	D3.2. User handbook of the Biobanking solution for PARASITE	3	CHG3	PU	food safety authorities			
WP3	D3.4. Biobank of parasite animals and biomolecules	36	CHG3	PU				
	Computer-aid epidemiological geo-referenced database for zoonotic parasites in fish stocks and products marketed in Europe						Comprehensible information on epidemiology	Handbook/user
	D3.3. User handbook and technical solutions for management of parasitized fish stocks and products	36	СНG3	RE	Public Authorities: (Health and fisheries) Industry		(data, photo gallery) manual, website access and presentations. industry in self control programmes	
	New genetic tools for identification and characterization of anisakid species and knowledge on the genetic variability of populations						Information on	Conference/ Symposia
WP4	D4.1. Standardized protocols for DNA extraction, PCR and sequencing of mtDNA cox2	3	UT-URS	RE	Scientific		new genetic tools and	Papers, Posters,
	D4.2. Genetic data (sequences, genetic analysis of the data gathered from different genetic data sets, population genetics, genetic diversity and variability estimates)	30	UT-URS	RE	Community		associated knowledge	Communications Scientific and technical reports

	Portfolio of transferrable/spreadable results					PARASITE 31				
	Result/	Available	Partners in	Dissem.	Target public		Type of activity	Material/ Format		
	Related Deliverable	month	charge	level*	Target public	Training	Dissemination	Material/ Torritat		
	Knowledge on characterization of allergens in fishery products									
- WP5	D5.1. Report on characterization of allergens in extractive fishing products and aquaculture fish.	22	SERMAS	RE	-			Conference/ Symposia Papers,		
	Knowledge on the allergenic capacity of Anisakidae worms other			Knowledge gained in allergens, allergenic capacity and immune response	Posters, Communications Scientific and technical reports					
	D5.2. Report on determination of the allergenic capacity of Anisakidae worms other than Anisakis species in an animal model	30	ISS	RE	- Scientific					
	Knowledge on allergens localization and relationship to genetic v	Community								
	D5.3. Report on allergens localization and relationship to genetic variability	30	CSIC-MNCN	RE	<ul> <li>and</li> <li>Health &amp; Food</li> <li>Authorities</li> </ul>		Knowledge gained in allergens, allergenic	Conference/ Symposia Papers, Posters, Communications		
	Knowledge on cellular and humoral responses to the anisakid parasites						capacity and immune response	Scientific and technical reports		
	D5.4. Report on cellular and humoral responses to the Anisakid parasites	33	SERMAS	RE						

	Portfolio of transferrable/spreadable results						Input for		
	Result/	Available	Partners in charge	Discom loval*	Target	Туре о	f activity	Material/ Format	
	Related Deliverable	month	Farmers III charge	Disseili. levei	public	Training	Dissemination	wateriai/ Format	
	Improved technological UV-based solutions for parasites screening	-	Scientific	Scientific	utilities on	New devices utilities and	Workshops Audiovisual		
	D6.1. Prototype based on UV-microtech to be used in SMEs	12	CSIC-IIM	RE	Community	(labs) Training for beta- testing (industry) Training for wider public (industry and inspection bodies)	previous state of	material	
WP6	D.6.2. Technical device to test the viability of anisakids in processed fish products	24	TNET	RE	Inspection bodies Industry			Presentations Catalogue of transferable technologies Project website	
WP6	Real Time-PCR based methodology to detect parasites and/or their traces	1	1	1				Workshops	

<sup>3</sup> For replicating the workshop in different locations without relevant additional cost, so that a great number of final users can be reached

							PAF	ASITE 312068
	Portfolio of transferrable/spreadable results						Input for	
	Result/	Available	Partners in charge	Dissem. level*	Target	Туре с	of activity	Material/ Format
	Related Deliverable	month	Partners in charge	Dissein. level	public	Training	Dissemination	Material/ Format
	Real Time-PCR based methodology to detect parasites and/or their traces			New devices				
	D6.3. Specific primers to implement existing molecular methods to detect anisakids and raphidascarid and their traces	24	UDRS	RE	Scientific Community	Harmonization (labs)	utilities and advantages over previous state of	Audiovisual material <sup>3</sup> Presentations
WP6	Immuno assays to detect parasites and/or their traces				Inspection bodies	Training for beta-	the art.	Catalogue of
	D6.4. Monoclonal antibodies for the detection of anisakids	36	CSIC-MNCN	RE	and Industry		All: UV-based solutions; RT PCR and Immune assays	transferable technologies
	Knowledge on the infectivity and inactivation of parasites in relation to treatments, host fish different hosts	species, an	d effects of passage	through	Scientific Community		Conference/ Symposia Scientific and Technical Journals	Papers, Posters,
	D.7.1. Report on viability and infectivity of parasites in fish and fishery products in relation to host species, and effects on passage through different hosts	20	CSIC-IIM	PU	Food & Health Authorities			Communications Scientific and technical reports
	D.7.2. Report on bacteria-parasite interaction	24	NIFES	PU	Autionities			
	Knowledge on optimal treatments to inactivate the parasite and its antigens in fishery produ	Scientific		_	Papers, Posters, Communications			
WP7	D.7.3. Report on treatments for killing parasites in fishery products	32	CSIC-ICTAN	PU	Community Food &	Prototype	Conference/ Symposia Scientific Journals Technical sectoral	Scientific and technical reports
	D.7.4. Report on antigen elimination or inactivation methods	32	CSIC-ICTAN	RE	Health	(Industry)		Workshops
	D.7.5. Prototype for management on board of parasite contaminants in residues	32	CSIC-IIM	RE	Authorities Industry		publications	Audiovisual material <sup>4</sup> Project website
	Knowledge for effectively reducing the risks related to fish parasites in the production chain	Consumers		Mass media Web: specialized	Press releases Contributions to			
	D.7.6. Guideline for parasite risk management in the food chain	36	CSIC-ICTAN	PU	Civil Society		blogsphere, relevant related social networks,	blogs and other open forums Leaflets Project website
WP8	Information on parasite abundance and genetic variability		•		Scientific		Conference/	Papers, Posters,
	D8.1. Report on statistical analysis of parasite abundance and genetic variability	28	UNIABDN	RE	Community		Symposia Scientific Journals	Communications Scientific and

<sup>4</sup> For replicating the workshop in different locations without relevant additional cost, so that a great number of final users can be reached

	Portfolio of transferrable/spreadable res	ults					Input for		
	Result/	Available	Partners in charge	Discom loval*	Target	Туре	of activity	- Material/ Form	
	Related Deliverable	month	Farmers in charge	Disseili. levei	public	Training	Dissemination		
	Information on parasite abundance and genetic variability	I		P					
	D8.1. Report on statistical analysis of parasite abundance and genetic variability	28	UNIABDN	RE	Scientific				
	Information on quantitative risk assesment				Community			technical reports Leaflets Project website	
	D8.2. Report on quantitative risk assessment	36	UNIABDN	PU			Technical sectoral publications		
P8	Information on consumer willingness to to pay for treatments to reduce incidence of par	rasites	1		Public Authorities: Health and				
	D8.3. Report on Willingness to Pay	B.3. Report on Willingness to Pay   36   UNIABDN   PU	listienes						
	Information on cost/benefit of the application of treatments and tools for reducing the right	sk related to par	asites	1	Industry				
	D.8.4. Report on Cost/Benefit scenarios Policy/Food producers	36	LARPRO	PU					

#### B3.2.2 Management of intellectual property

As a general basic rule for IPR management in this project, foreground knowledge will be owned by those partners directly involved in its achievement.

Regardless other unexpected results, PARASITE consortium members have pre-identified the results susceptible for different protection strategies. To definitely the most suitable strategy in each case, it has been set up and advisory team on IPR and the Coordinator will be supported by a company specialised in providing IPR support.

The Consortium agreement will also establish the agreed rules for IPR management in the project, including the consideration of access rights to relevant background knowledge own by partners.

Special care will be taken to prevent partners from disclosure of any information regarding results susceptible for IPR protection.

Besides this, documents and other contents resulting from project deliverables, etc. will be copyright registered when relevant.

RESULT SUSCEPTIBLE FOR PROTECTION	KIND OF PROTECTION <sup>5</sup>	COMMENTS
WP3: <b>Biobank</b> software (tentatively under the trademark PARASITE-Bank)	Registration of copy right and possibly of a trademark. Intellectual property protection.	Owner: CHG3
WP4: <b>Primers</b> to be used as DNA barcodes	Patent	Owner: UT-URS
WP6: Immune assays based on <b>monoclonal antibodies</b> to detect anisakids' antigens in fishery products	Patent	Owners: CSIC and SERMAS
<ul> <li>WP6: Device to assess viability of anisakids in fishery products (1)</li> <li>Device designed for mass and fast application of UV-press method to detect anisakids (2)</li> </ul>	2 patents	Owners: 1. CSIC, NIFES 2. Technet & MRI
<ul> <li>WP7: 1. Method for Anisakis antigen elimination in fishery products (SERMAS)</li> <li>2. Device to inactivate or kill parasites on Board (Spinboard): offals and discards.</li> </ul>	2 patents	1. SERMAS 2. CSIC, CETMAR

In any case, PARASITE will not act in contradiction with the rules laid down in Annex II of the GA.

# **B4.Ethical issues**

## General

- 1. The ethical standards and guidelines compatible with, and equivalent to, those of FP7 will be rigorously applied, regardless of the country in which the FP7 funded research is carried out.
- 2. Detailed information will be provided to the relevant national/local/institutional Ethics Committees in relation to the organization of surveys and interviews concerning the recruitment strategies, the informed consent procedures that need to be followed and the data handling and privacy protection safeguards, as follow: Human samples

In WP5 some tasks require use of human sera. These will be obtained in informed consent to blood drawing, human sera will be collected from donors. All samples will be obtained with prior informed consent. Before collecting samples, patients will be asked about their willingness to participate in the study, will be informed of the aims of the study and the consequences of analysis. It will be made clear that samples will be anonymized and that only the treating physician will hold a code for patient identity information, and that if the patient is not willing to take part in the study, this will not cause any prejudice as to future treatment or care. Further, the patient will be informed that will be able to withdraw her/his samples from the study at any time during the course of the project, that the samples will then be destroyed, and this will have no consequences for future treatment or care. The patient will also be informed as to how the results of the study will be disseminated (through scientific peer-reviewed journals). The patients should also understand that no direct benefits will derive from the study for the patient her/himself.

#### **Data Protection Issues**

All clinical data and samples will be anonymized before data entry into the sample database, which is done by the treating clinician. Only treating physicians will be collecting clinical and demographic data code numbers will be kept separate from clinical details in accordance with local data protection regulations. Age-and sex-matched controls will also be recruited. Patients will undergo a single interview, clinical examination and blood sampling. Controls will be seen once and will also be interviewed regarding clinical conditions before donating a blood sample. After this, the patients and controls will have completed their contribution to the study.

The results of the project research studies will be reported only in summary form and it will not be possible to give patients or controls their own individual results except for any routine laboratory tests done as part of the clinical assessment. The participants will conform to relevant EU legislation, including the Charter of Fundamental Rights of the EU and the Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data. We will also comply with Directive 98/44/EC of the European Parliament and of the Council of 6 July 1998 on the legal protection of biotechnological inventions, international conventions and declarations. All participants will comply with the following international conventions and declarations: the Helsinki Declaration in its latest version, Convention of the Council of Europe on Human Rights and Biomedicine signed in Oviedo on 4 April 1997, and the Additional Protocol on the Prohibition of Cloning Human Beings signed in Paris on 12 January 1998. We will also comply with the Universal Declaration on the human genome and human rights adopted by UNESCO.

Projects involving interview surveys raise some date protection and confidentiality issues. The data collected may include personal social and/or economic information, e.g. sex, age, employment, income category, previous exposure to seafood parasites and the project questionnaires will examine attitudes, beliefs and perceptions in relation to seafood products. Information collected specifically excludes any other aspects of health, sexual lifestyle, ethnicity, political opinion, religious and philosophical conviction or criminal records. The data will be collected from consenting adults

only. Interviewees will be informed of the purpose of the surveys and participation will be entirely voluntary. A questionnaire (for Spain and UK) will be put to a 1000 individuals randomly selected from the population as well as 200 people from high risk group. This latter group is to include individuals who have been affected by fish-borne parasites. To identify these individuals we will get the help of the appropriate health agencies (Health Protection Scotland and the Ministry of Health and Social Policy in Spain). These organisations will write to these individuals on our behalf and with their consent we will bring them into the study. This part of the work will require ethical approval from the appropriate University Ethical Committees, notwithstanding that the ethical requirement of informed consent will be observed during all stages of the survey. A protocol will be written and the appropriate ethics forms will be sent to the local UK NHS REC and local Spanish National Health System (Research Ethic Committees). We also require having notification on the numbers of people reporting ill with fish-borne parasites. This we can get as aggregated data from the health-protection agencies so there would be no need to get ethical approval for this, however we will include it in our protocol above so that the ethics committee can see the extent of our study. In Task 8.4 there is another survey re willingness to pay. For this part of the work ethical approval from the appropriate University Ethical Committees will be sought. However, it should be also noticed that the ethical requirement of *informed consent* will be observed during all stages of the survey.

3. Copies of local/national approval of the Ethics Committees from each country where the studies involving human participants will take place will be forwarded to the European Commission prior to the beginning of the relevant research.

In PARASITE, the Ethics approval for the study with human participants will be provided by the Ethics Committee of SERMAS (Partner 6). All subjects taking part in the study will provide written informed consent to participate (the form is enclosed).

The informed consent form will meet the requirements of Spanish Regulations for Biomedical Research (Law 14/2007) and for biorepositories (Order 1716/2011). The informed consent form consists of two parts: the information sheet and the consent certificate. The informed consent form includes issues on treatment of data and confidentiality, destination of samples beyond the lifetime of the project, benefit and medical attention and access to samples and/or information. The Law 14/2007 is built on the principles of integrity of persons and protection of the dignity and identity of human beings in any research involving biomedical interventions on humans, as well as when genetic analysis is performed, the treatment of personal genetic data and biological samples of human origin to be used in research. The Order 1716/2011 published on 18 November 2011, establishes the basic requirements for authorization and operation of biorepositories in biomedical research and treatment of biological samples of human origin, and regulates the operation and organization of the National Register of Biorepositories for biomedical research.

 The Project will comply with the new directive 2010/63/EU regarding "the protection of animals used for scientific purposes", which will apply to their research from January 1<sup>st</sup>, 2013. A 3Rs principle (Replacement, Reduction, and Refinement) will also be applied in animal experiments when possible.

The Replacement (the use of non-animal methods, such as cell cultures or other yet available invitro models) is not applicable throughout the animal experimental design described in the project. Since there is not any available in vitro model to study the infectivity of Anisakidae larvae, larval infectivity will be tested in animal models. The Reduction principle can be quite applicable since the number of animals will keep to a minimum enabling to obtain comparable amounts of information from fewer animals (it will be obtain the most possible information from the same number of animals). The Refinement principle will be applied to the breeding, the accommodation and the care. Procedures will be carried out under general or with local anesthesia; instead procedures involving severe pain, suffering or long-lasting distress that cannot be ameliorated will be avoided. In all cases, animals will be humanely killed by methods that are considered to be acceptable in the directive 2010/63 Annex IV.

5. Copies of local/national of the Ethics Committees and authorization from animal experiment will be forwarded to the European Commission prior to the initiation of animal experiments. Biosecurity and bioethics reports for animal experiments will be provided to the European Commission by the partners in charge of the tasks involving animal trials.

These authorizations will be supervised by Ethics Committee members with expertise on animal research under new directive 2010/63/EU. Laboratory animals (mice, rats or hamsters and fish) will be housed and treated according to the European Directive on laboratory animal welfare (Directive 2010/63/EU).

Copies of training certificates of the staff involved in animal experiments will be forwarded to the Commission together with the Ethics approval and authorization approval forms prior to the initiation of animal experiments.

6. All staff, including those in ICPC, handling infectious or chemical agents, staff directly involved in parasite data recording and fishing will be adequately informed (bio-security training) about the project's objectives, methodology, potential health risk (exposure) and necessary safety measures. Training on bio-security and lab safety will be a precondition for starting work at each workpackage.

A project internal training workshop will be organized during the first six months after project start where key technical personnel from every partner organization/institution will be invited to participate. During the workshop, safety guidelines related to the handling and processing of infectious or chemical agents, parasite data recording and fishing will be addressed. Additionally, a manual summarizing the operational and safety aspects of the methods and procedures will be prepared and distributed to the staff of every partner involved in the sample and data compilation. In any case, safety guidelines concerning the protection of health and safety at work under the EU directive 2000/54/CE will be addressed.

#### 7. Biological sample collection in ICPC countries:

The project will source seafood (fish and squid) products from ICPCs. However, these are products which are already commercially imported into the EU and therefore involve no additional legal or health issues. Nevertheless, the project will consider the provision of fair benefit sharing arrangements with ICPC partners and effectively manage this during the project, to ensure that procedures will be implemented to facilitate effective capacity building.

# 8. Clarification why certain dissemination activities are restricted and assuring communication with public authorities and the broader society':

The Exploitation and Dissemination Committee (as the communication branch of the Project) will disseminate the relevant findings on Ethic issues to the appropriate health authorities in the member states participating in this project. Furthermore, Ethics information will be forwarded to the European Food Safety Agency and the Community Reference Lab for Parasites. Additionally, the information will also be sent to the Food Standards Agency. In all cases, the communication will be done by pro-active meetings/presentations.

As explained above (B3.2.1.), in a broader sense the restriction for dissemination activities of some results must follow the basic rules for IPR management in this project, Additionally, some restricted level will only be applied to targeted dissemination actions that can raise unwarranted consumer alarms or harm seafood producers, and as a consequence a risk on reduced consumption of seafood.

9. The project has no foreseeable harmful effect on the environment or abnormal safety aspects. In contrast, improved understanding and treatment of zoonotic parasites in seafood should improve food safety.

## ETHICS ISSUES TABLE

(Note: Research involving activities marked with an asterisk \* in the left column in the table below will be referred automatically to Ethical Review).

	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 PROPOSAL	х	

	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?	Х	WP5
	Does the proposed research involve Human data collection?	Х	WP8
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY		
	PROPOSAL		

Privacy	YES	Page
Does the proposed research involve processing of genetic informat personal data (e.g. health, sexual lifestyle, ethnicity, political opinion religious or philosophical conviction)?		WP8
Does the proposed research involve tracking the location or observ people? I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		

Resea	rch on Animals	YES	Page
Does the proposed research invo	lve research on animals?	X	WP7
Are those animals transgenic sm	all laboratory animals?		
Are those animals transgenic far	n animals?		
Are those animals non-human pr	mates?		
Are those animals cloned farm a	nimals?		
I CONFIRM THAT NONE OF TH	E ABOVE ISSUES APPLY TO MY		
PROPOSAL			

	PARASI	TE 312068
Research Involving ICP Countries	YES	Page
Is the proposed research (or part of it) going to take place in one or more ICP Countries?		
Is any material used in the research (e.g. personal data, animal and/or human tissue samples, genetic material, live animals, etc):		
a) Collected in any of the ICP countries?		
b) Exported to any other country (including ICPC and EU Member States)?		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	Х	

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 PROPOSAL	х	

# **B4. Consideration of gender aspects**

There is currently a gender stable balance between the scientists participating in the field of Marine Parasitology and Food Technology related with seafood parasites that is also encompassed by the PARASITE proposal. In fact, over 40 % of the RTD persons involved in this proposal are women scientists (45% of WPs leaders), some of them with key roles in the proposal.

Because some partners have still to allocate resources to non-identified persons (technicians, Phd, post-doc), the recruitment of contracts will be without gender bias, following established good practice for recruitment and consistent with relevant national and European laws and directives on avoidance of discrimination on the basis of sex, religion, sexual orientation, etc. We will do our best to encourage applications from woman researcher and doctoral students, although the overriding criterion will be based on merit. Selection panels will include female researchers. Consideration of family commitments and flexible working practices will be made in the running of the project and in the organization of consortium events.

In accordance to Articles 2 and 3 of the Treaty of Amsterdam (1997) and other EU Directives the consortium is committed to incorporate the principles of gender mainstreaming throughout the various elements of the project. At a practical operational level, the Project Manager will ask each team leader to report on employments practices in each institution and will endeavour to promote European best practice across the PARASITE Consortium.

We plan to considerably improve this situation by strongly encouraging female candidate to apply for the open positions in the project, and thus contribute towards the goal of reaching gender equality throughout the course of the project. The following objectives underpin the gender action plan:

• Ensuring that women and men have equal opportunities to participate in the various parts of the project

• In addressing diversity, the work plan will take account of the different situations needs and interests of women and men.

• The work plan will contribute to reducing inequalities between women and men.

The Coordinator will monitor developments and should any specific gender issues arise, it will be managed in the Steering Committee.

## **B6.** References

Arizono, N., 2011. Human infection with Pseudoterranova azarasi roundworm. Emerg. Infect. Dis. 17, 555-556.

Audicana M.T. and Kennedy, M.W., 2008. *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. *Clinical Microbiology Reviews* 21, 360–379

Bererciartua Achaga, J.A., 2005. Patente ES 2 213 486 (B1) Procedimiento para eliminar parásitos en pescado

Chai, J.Y.K., Murrell, D. and Lymbery, A.J., 2005. Fish-borne parasitic zoonoses: status and issues. *Int J Parasitol* 35, 1233–1248.

Chern, W.S., Rickertsen, K., Ssuboi, N. & Fu, T.-T., 2002. Consumer acceptance and willingness to pay for genetically modified vegetable oil and salmon: a multiple-country assessment. *AgBioForum* 5, 105-112.

D'Amelio S, Mathiopoulos KD, Santos CP, Pugachev ON, Webb SC, Picanço M, Paggi L 2000: Genetic markers in ribosomal DNA for the identification of members of the genus *Anisakis* (Nematoda: Ascaridoidea) defined by polymerase chain reaction-based restriction fragment length polymorphism. *Int J Parasitol*, 30:223-226.

Espiñeira, M., Herrero, B., Vieites, J. M., and Santaclara, F. J. (2010). Detection and identification of anisakids in seafood by fragment length polymorphism analysis and PCR-RFLP of ITS-1 region. *Food Control*, 21, 1051-1060.

Fagerholm HP, 1998. Incubation in rats of a nematodal larva from cod to establish its specific identity: *Contracaecum osculatum*, (Rudolphi). *Parasitol. Res.*, 75, 57-63, 1988.

Herrero, B., Vieites, M and Espiñeira, M, 2011 Detection of anisakids in fish and seafood products by real-time PCR. *Food Control*, Volume 22, (6) 933-939

Kliks, 1983; Kliks, M.M., Anisakiasis in the Western United States: four new case reports from California. *Am. J. Trop. Med. Hyg.*, 32, 526, 1983

Karl, H., Leinemann, M., 1993, A fast and quantitative detection method for nematodes in fish fillets and fishery products. *Arch. Lebensmittelhyg* 44, 105-128.

Lee, A. et al., 1985. A case of human infection with the larva of Terranova type A. Korean J. Pathol., 19, 463. [In Korean].

Levsen, A. and Lunestad, B.T. 2010. *Anisakis simplex* third stage larvae in Norwegian spring spawning herring (*Clupea harengus* L.), with emphasis on larval distribution in the flesh. *Veterinary Parasitology* 171: 247-253.

Lindqvist R and Westoo A. 2000. Quantitative risk assessment for *Listeria monocytogenes* in smoked or gravad salmon and rainbow trout in sweden. *Int J Food Microbiol.* 58(3):181-96.

Lopez I and Pardo MA, 2010. Evaluation of a real-time polymerase chain reaction (PCR) assay for detection of Anisakis simplex parasite as a food-borne allergen source in seafood products. *J Agric Food Chem.* 58(3):1469-77

Mattiucci S and Nascetti G. 2006. Molecular systematics, phylogeny and ecology of anisakid nematodes of the genus *Anisakis* Dujardin, 1845: an update. *Parasité*, 13:99-113.

Mattiucci, S. and Nascetti, G. 2008 Advances and trends in the molecular systematics of Anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. *Advances in Parasitology*, 66, 47-146.

Mattiucci S., Nascetti G., Dailey M., Webb S.C., Barros N., Cianchi R., Bullini L. 2005. Evidence for a new species of the genus *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae): morphological description and genetic relationships between congeners. *Systematic Parasitology*, 61: 157-171.

Mattiucci S., Paoletti M., Webb S.C., Sardella N., Timi J.T., Berland B., Nascetti G. 2008. Genetic relationships among species of *Contracaecum* Railliet & Henry, 1912 and *Phocascaris* Host, 1932 (Nematoda: Anisakidae) from pinnipeds based on mitochondrial *cox2* sequences, and congruence with allozyme data. *Parasite, Journal de la Societe Française de Parasitologie*, 15: 408-419.

Mattiucci S, Paoletti M, Webb SC 2009: *Anisakis nascettii* n. sp. (Nematoda: Anisakidae) from beaked whales of the southern hemisphere: morphological description, genetic relationships between congeners and ecological data. *Syst Parasitol*, 74:199-217.

Mattiucci S., Paoletti M., Borrini F., Palumbo M., Macarone Palmieri R., Gomes V., Casati A., Nascetti G. 2011a. First molecular identification of the zoonotic parasite *Anisakis pegreffii* (Nematoda: Anisakidae) in a paraffin-embedded granuloma taken from a case of human intestinal anisakiasis in Italy. *BMC Infectious Diseases*, 11: 82.

Mattiucci S., Paoletti M., Webb CS, Nascetti G 2011b. *Pseudoterranova* and *Contracaecum*. Invited Chapter In: *Molecular detection of human parasitic pathogens*. (Don Liu Editor). CRC Press, Boca Raton, FL, USA, (in press).

McNab WB. 1997. A literature review linking microbial risk assessment, predictive microbiology and dose-response modelling. *Dairy Food Environ Sanit.* 17:405-16.

Mossali C, Palermo S, Capra E, Piccolo G, Botti S, Bandi C, D'Amelio S, Giuffra E.Sensitive detection and quantification of anisakid parasite residues in food products. *Foodborne Pathog Dis.* 2010;7(4):391-7

Nadler SA, D'Amelio S, Dailey MD, Paggi L, Siu S, Sakanari JA 2005: Molecular phylogenetics and diagnosis of *Anisakis, Pseudoterranova*, and *Contracaecum* from northern pacific marine mammals. *J Parasitol* 2005:1413-1429.

Nagasawa, K and Moravec, F. 1995. Larval anisakid nematodes of Japanese common squid (*Todarodes pacificus*) from the Sea of Japan. *J. Parasitol.* 81(1): 69-75.

Petrie, A., Wootton, R., Bruno, D., Mackenzie, K., Bron, J., 2007. Survey of *Anisakis* and *Pseudoterranova* in Scottish fisheries and the efficacy of current detection methods. Final Report on FSAS Project S14008. Institute of Aquaculture, University of Stirling, Stirling

Rodríguez-Mahillo AI., González-Muñoz M, de las Heras C, Tejada M, Moneo I., 2010. Quantification of *Anisakis simplex* allergens in fresh, long-term frozen and cooked fish muscle. *Foodborne Pathogens and Disease*, 7(8), 967-973.

Rodriguez-Mahillo, A.I., Gonzalez-Munoz, M., Moneo, I., Solas, M.T., Mendizabal, A., de las Heras, C., Tejada, M., 2008, Allergenic properties and cuticle microstructure of *Anisakis simplex* L3 after freezing and pepsin digestion. *J Food Prot* 71, 2578-2581.

Shamsi S. and Butcher AR. 2011. First report of human anisakidosis in Australia. *Med. J. Aust* 21:199-200.Suzuki, Mrata R., Hosaka M., Araki J. 2010. Risk factors for human *Anisakis* infection and association between the geographic origins of *Scomber japonicus* and anisakid nematode. *Int. J. Food Microbiol.*, 137: 88-93.

Skov J., Kania P., Dalsgaard A., Jørgensen T.R., Buchmann K., 2009. Life cycle stages of heterophyid trematodes in Vietnamese freshwater fishes traced by molecular and morphometric methods. *Vet. Parasitol* 160: 66-75.

Solas MT, García ML, De las Heras C., Rodríguez-Mahillo AI, González-Muñoz M., Moneo, I., Mendizábal A., Tejada M. 2009. *Anisakis simplex* antigens in fresh and frozen thawed muscle of anchovies in vinegar. *Food Sc. Tech. Int.* 15; 139-148,

Suzuki, Mrata R., Hosaka M., Araki J. 2010. Risk factors for human *Anisakis* infection and association between the geographic origins of *Scomber japonicus* and anisakid nematode. *Int. J. Food Microbiol.*, 137: 88-93

Tejada M, Solas MT, De las Heras C, Rodríguez-Mahillo AI, González-Muñoz M, Moneo I, Mendizábal A. 2007. Antigenic activity of *Anisakis* larvae is conserved after food processing and pepsin treatments. *Parassitologia* 49(2) 406

Thuy D.T., Kania P., Buchmann K., 2010. Infection status of zoonotic trematode metacercariae in Sutchi catfish (*Pangasianodon hypophthalmus*) in Vietnam: Associations with season, management and host age. *Aquaculture*302:19-25. Torres P. et al., 2007. Human pseudoterranovosis, an emerging infection in Chile.J. *Parasitol.*, 93, 440-443.

Umehara, A., Kawakami, Y., Araki, J., Uchida, A. 2007. Molecular identification of the etiological agent of the human anisakiasis in Japan. *Parasitology International*, 56(3), 211-215.

Valentini A, Mattiucci S, Bondanelli P, Webb SC, Mignucci-Giannone A, Colom-Llavina MM, Nascetti G 2006: Genetic relationships among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial *cox-2* sequences, and comparison with allozyme data. *J Parasitol*, 92:156-166.

Vidaček, S. De las Heras C. Solas MT. Tejada M., 2009. Effect of high hydrostatic pressure on mortality of *Anisakis simplex* L3 and on muscle properties of infested hake. *J Sci Food Agric* 89: 2228–2235

Vidaček, S. De las Heras C. Solas MT, Mendizábal, A., Rodríguez-Mahillo AI, Tejada M., 2010. Antigenicity and Viability of *Anisakis* larvae heated at different time-temperature conditions *J Food Prot* 73(1): 62-68. 2010

Vose D. 2000. Risk analysis: A quantitative guide. 2nd Edition ed. Chichester, England, UK: John Wiley & Sons.

Yu, J.R. et al., 2001. A human case of gastric infection by Pseudoterranova decipiens larva.Korean J. Parasitol., 39, 193.

Zuur, A. F., Ieno, E. N., and Elphick, S. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1: 3-14.

Zuur AF, Ieno EN, Smith GM. 2007. Analysing ecological data. Springer, New York. 672 pp.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., and Smith, G. M. 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, Berlin. 574 pp.

Zuur AF, Saveliev AA & Ieno EN, 2012. Zero Inflated Models and Generalized Linear Mixed Models with WinBUGS and R

#### Consortium experience (enlarged reference list)

- Barletta, B. 2007. Biochemical and molecular biological aspects of silverfish allergens. Protein Pept. Lett. 14,970-974.

- Capobianco, F. 2008. Oral sensitization with shrimp tropomyosin induces in mice allergen-specific IgE, T cell response and systemic anaphylactic reactions. Int. Immunol. 20,1077-1086.

- Chaligiannis, I. 2012. Anisakidae infection in fish of the Aegean Sea.Vet. Parasitol. 184, 362-366.

- Karl, H. 2008. Nematode larvae in fish on the German market - 20 years of consumer related research. Archiv Lebensmittelhyg. 59,107-116.

- Karl, H.; Baumann, F.; Ostermeyer, U.; Kuhn, T.; Klimpel S. 2011. *Anisakis* nematode larvae in wild caught salmon species of Alaska and possible post-mortem migration. Diseases of Aquatic Organisms (Dao) 94, 201-209.

- Karl, H.; Levsen, A. 2011. Occurrence and distribution of anisakid nematodes in Grey gurnard (*Eutrigla gurnardus* L.) from the North Sea.Food Control 22, 1634 – 1638.

- Karl, H.; Meyer, C.; Banneke, S.; Sipos, G.; Bartelt, E.; Lagrange, F.; Jark, U.; Feldhusen, F. 2002. The abundance of nematode larvae *Anisakis* sp. in the flesh of fishes and possible post-mortem migration. Archiv Lebensmittelhyg. 53,118-120.

- Klimpel, S.; Kuhn, T.; Busch, M.W.; Karl, H.; Palm, H.W. 2011. Deep-water life cycle of *Anisakis paggiae* (Nematoda: Anisakidae) in the Irminger Sea indicated kogiid whale distribution in north Atlantic waters. Polar Biol. 34, 899 – 906

- Lalle, M. 2009. High genetic polymorphism among *Giardia duodenalis* isolates from Sahrawi children. Trans. R. Soc. Trop. Med. Hyg. 103, 834-838.

- Lalle, M. 2011. Dematin, a component of the erythrocyte membrane skeleton, is internalized by the malaria parasite and associates with Plasmodium 14-3-3. J. Biol. Chem. 286, 1227-1236.

- Lalle, M. 2011. Expression of *Cryptosporidium parvum* Cpa135/CpCCP1 chimeras in *Giardia duodenalis*: organization of the protein domains affects the protein secretion pathway. Exp. Parasitol. 127, 680-686.

- Lalle, M. 2011. *Giardia duodenalis* 14-3-3 protein is polyglycylated by a tubulin tyrosine ligase-like member and deglycylated by two metallocarboxypeptidases. J. Biol. Chem. 286, 4471-4484.

- Mastrangeli, G. 2009. Effects of live and inactivated VSL#3 probiotic preparations in the modulation of in vitro and in vivo allergen-induced Th2 responses. Int. Arch. Allergy Immunol. 150, 133-143.

- Murrell, K.D. 2011. Worldwide occurrence and impact of human trichinellosis, 1986-2009. Emerg. Infect. Dis. 17, 2194-2202.

- Schiavi, E. 2011. Oral therapeutic administration of a probiotic mixture suppresses established Th2 responses and systemic anaphylaxis in a murine model of food allergy. Allergy. 66, 499-508.

- Strachan, N. J. C., C. J. Hunter, C. D. R. Jones, R. S. Wilson, S. Ethelberg, P. Cross, A. P. Williams, O. Rotariu, and D. Chadwick. 2011. The relationship between lay and technical views of *Escherichia coli* O157 risk. Philos T Roy Soc B. 366, 1573, 1999-2009.

-"Confronting Objections to Performance Pay: A Study of the Impact of Individual and Gain-sharing Incentives on the Job Satisfaction of British Employees", (with K. Pouliakas), <u>Scottish Journal of Political Economy</u>, 56, 662-684, 2009.

-"Job satisfaction and Target Earnings" (with S. Drakopoulos), Journal of Economic Psychology, 18, 693-704, 1997.

-"Jobs as Lancaster Goods: The Determinants of Overall Job Satisfaction and its Composition" (with A. Skalli and E. Vasileiou), Journal of Socio-Economics, 37, 1906–1920, 2008.

-"Measuring the Utility Cost of Temporary Employment Contracts using a Conjoint Analysis Approach" (with K. Pouliakas), <u>Economica</u>, 77, 688-709, 2010.

-"Socio-economic Differences in the Perceived Quality of High and Low-paid Jobs in Europe", (with K. Pouliakas), <u>The</u> International Labour Review, 149, 1, 29, 2010.

-"The Economics of Health and Safety at Work: An Interdisciplinary Review of the Theory and Policy", (with K. Pouliakas), Journal of Economic Surveys, forthcoming, 2011.

-"The Effects of Low Pay and Unemployment on Psychological Well-Being: A Logistic Regression Approach", <u>Journal of</u> <u>Health Economics</u>, 17, 85-104, 1998.

-"Willingness for Mobility Amongst European Fishermen" (C. Pita, H. Dickey, G. J. Pierce, E. Mente), Journal of Rural Studies, 26, 308-319, 2010.

-Arcos, S.C., González Muñoz, M., Mendizabal, A., Moneo, I., Navas, A., Robertson, L., Tejada, M. 2012. *Anisakis*. (A. Navas coord.). Informes CSIC, Consejo Superior de Inevstigaciones Científicas (in press 2012).

-Armignacco, O. 2008. Human illnesses caused by *Opisthorchis felineus* flukes, in Italy. Emerg. Infect. Dis. 14, 1902-1905. -Beauchamp K.A., El-Matbouli M., Gay M., Georgiadis M.P., Nehring R.B., Hedrick R.P. 2006. The effect of cohabitation of Tubifex tubifex (Oligochaeta: Tubificidae) populations on infections to Myxobolus cerebralis (Myxozoa: Myxobolidae).

Iubitex tubitex (Oligochaeta: Iubiticidae) populations on infections to Myxobolius cerebralis (Myxozoa: Myxobolidae). Journal of Invertebrate Pathology 91:1-8

-Calvo, E., Flores-Romero, P., López, J.A. and Navas, A. 2005. Identification of proteins expressing differences among isolates of *Meloidogynespp*. (Nematoda: Meloidogynidae) by nano-liquid chromatography coupled to ion-trap mass spectrometry. *Journal of Proteome Research* 4, 1017-1021.

-Casulli, A. 2012. Genetic variability of *Echinococcus granulosus* sensu stricto in Europe inferred by mitochondrial DNA sequences. Infect. Genet. Evol. 12, 377-383.

-Chang Kangmei, Li Huan, Lü Zhenming, Chi Changfeng.2010.Genetic variation in different populations of *Octopus variabilis* in China coastal waters based on the COI gene analysis. Oceanologia ET Limnologia Sinica, 41(3):308-314.

-Chettri, J. K., Holten-Andersen, L., Raida, M. K., Kania, P., Buchmann, K. (2011) PAMP induced expression of immune relevant genes in head kidney leukocytes of rainbow trout (*Oncorhynchus mykiss*). Developmental and Comparative Immunology 35: 476-482

-Copin S., Robert-Pillot A., Malle P., Quilici M.L., Gay M. 2011. Evaluation of most-probable-number–PCR method with internal amplification control for the counting of total and pathogenic Vibrio parahaemolyticus in frozen shrimps. J Food Prot. 2012 Jan;75(1):150-3

-Di Felice, G. 2008. Use of probiotic bacteria for prevention and therapy of allergic diseases: studies in mouse model of allergic sensitization. J. Clin. Gastroenterol. 42, 130-132.

Fernández, I. C., Bohmer, K., Gallardo, J. M., Barros-Velázquez, J., Cañas, B. and Calo-Mata, P. 2010. Differential characterization of biogenic amine-producing bacteria involved in food poisoning using MALDI-TOF mass fingerprinting. Electrophoresis 31, 1116–1127.

-Bohmer, K., Fernández, I. C., Barros-Velázquez, J., Gallardo, J. M., Calo-Mata, P. and Cañas, B. 2010. Species differentiation of seafood spoilage and pathogenic gram-negative bacteria by maldi-tof mass fingerprinting. Journal of Proteome Research 9, 3169–3183.

-Bohmer, K., Fernández, I. C., Gallardo, J. M., Calo-Mata, P. and Cañas, B. 2011. Safety Assessment of Fresh and Processed Seafood Products by MALDI-TOF Mass Fingerprinting. Food Bioprocess Technol. 4, 907–918. -Fernández, I. C., Bohmer, K., Calo-Mata, P., Gallardo, J. M., Cañas, B. and Barros-Velázquez, J. 2012. Isolation and characterization of Streptococcus parauberis from vacuum-packaging refrigerated seafood products. Food Microbiology 30, 91-97.

-Bohmer, K., Fernández, I. C., Barros-Velázquez, J., Gallardo, J. M., Cañas, B. and Calo-Mata, P. 2011. Rapid species identification of seafood spoilage and pathogenic Gram-positive bacteria by MALDI-TOF mass fingerprinting.Electrophoresis 32, P. -W., Olsen, M. M., Lauridsen, J. H., Buchmann, K. (2009). Nematode infections of maricultured and wild fishes in 2951–2965.

-Fumarola, L. 2009. *Anisakis pegreffi* etiological agent of gastric infections in two Italian women. Foodborne Pathog. Dis. 6, 1157-1159.

-Gay M., Okamura B., de Kinkelin P. 2001. Evidence that infectious stages of Tetracapsula bryosalmonae for rainbow trout, Oncorhynchus mykiss, are present throughout the year. Diseases of Aquatic Organisms, 46: 31-40

-Gestal, C., Pascual, S., Guerra, A. 2007. *Aggregata octopiana* (Protista: Apicomplexa): a dangerous pathogen during commercial *Octopus vulgaris* ongrowing. *ICES Journal of Marine Science*, doi: 10.1093/icesjms/fsm154

-Gómez-Morales, M.A. 2008. Allergenic activity of *Molicola horridus* (Cestoda, Trypanorhyncha), a cosmopolitan fish parasite, in a mouse model. Vet. Parasitol. 157:314-320.

-Gómez-Morales, M.A. 2008. Validation of an enzyme-linked immunosorbent assay for diagnosis of human trichinellosis. Clin. Vaccine Immunol. 15,1723-1729.

-Gómez-Morales, M.A. 2009. International ring trial to detect anti-*Trichinella* IgG by ELISA on pig sera. Vet Parasitol. 166, 241-248.

-González, A.F., S. Pascual, C. Gestal, E. Abollo and A. Guerra. 2000. Influence of biotic and abiotic factors on parasitic infections of cephalopods in Galician waters. Fisheries Research, 60: 1877-183. -Vecchione, M., R.E. Young, A. Guerra, D.J. Lindsay, D.A. Clague, J.M. Bernhard, W.W. Sager, A.F. González, F. Rocha and M. Segonzac. 2001. Worldwide observations of remarkable deep-sea squids. Science, 294: 2505. -Otero, J., Salgado, X.A., González, A.F., Groom, S.B., Miranda, A., Cabanas, J.M. & Guerra, A. 2008. Bottom-up control of common octopus Octopus vulgaris in the Galician upwelling system, northeast Atlantic Ocean. Marine Ecology Progress Series, 362: 181-192.

-Herrero A.M., P. Carmona, M.L. Garcia, M.T. Solas, and M. Careche, Ultrastructural changes and structure and mobility of myowater in frozen-stored hake (*Merluccius merluccius* L.) muscle: Relationship with functionality and texture, J. Agric. Food Chem., 53, 2558-2566 (2005).

-Herrero, A.M., P. Carmona, and M. Careche, Raman spectroscopic study of structural changes in hake (*Merluccius merluccius* L.) muscle proteins during frozen storage, J. Agric. Food Chem., 52, 2147-2153 (2004).

-Jørgensen, L.v.G., Buchmann, K. (2011). Cysteine proteases as potential antigens in antiparasitic DNA vaccines. Vaccine 29, 5575-5583.

Karl, H. & Levsen, A. 2011. Occurrence and distribution of anisakid parasites in Grey gurnard (*Eutriglia gurnardus* L.) from the North Sea.*Food Control* 22, 1634-1638.

-Kroeger, М., Image processing. In: H.Rehbein, J.Oehlenschläger (Eds.), Wiley-Blackwell, Fishery Products Quality, safety and authenticity, Chichester, 2009 -Kroeger, M., Manthey-Karl, M. Tejada, M., Physical measurements, In: Kent, M., Knöchel, R., Barr, U.-K., Tejada, M., Nunes, L., Oehlenschläger, J. (Eds.), SEQUID – A new Method for Measurement of the Quality of Seafood, Shaker, Aachen, 2005

-Kroeger, M., Image analysis for monitoring the quality of fish, In: Luten, J.B., Oehlenschläger, J., Olafsdottir, G. (Eds.), Quality of Fish from Catch to Consumer, Labelling, Monitoring and Traceability, Wageningen Academic, Wageningen, 2003 -Gielsdorf, F., Rietdorf, A., Gruendig, L., A Concept for the Calibration of Terrestrial Laser Scanners, In: FIG Working Week 2004, Athens, Greece, 2004

-La Rosa, G. 2012. Development of a single larva microsatellite analysis to investigate the population structure of *Trichinella spiralis*. Infect. Genet. Evol. 12, 369-376.

-Levsen, A. & Jakobsen, P.J. 2002. Selection pressure towards monoxeny in *Camallanus cotti*(Nematoda, Camallanidae) facing an intermediate host bottleneck situation.*Parasitology* 124, 625-629. -Levsen, A. & Lunestad, B.T. 2010. *Anisakissimplex* 3<sup>rd</sup> stage larvae in Norwegian spring spawning herring (*Clupea* 

-Levsen, A. & Lunestad, B.T. 2010. *Anisakissimplex* 3<sup>rd</sup> stage larvae in Norwegian spring spawning herring (*Clupea harengus* L.), with emphasis on larval distribution in the flesh. *Vet. Parasitol.* 171, 247-253.

-Levsen, A., Lunestad, B.T. & Berland, B. 2005. Low detection efficiency of candling as a commonly recommended inspection method for nematode larvae in the flesh of pelagic fish. *J. Food Protect.* 68, 828-832.

-Li Huan, Lü Zhenming, Chang Kangmei, Chi Changfeng, Wu Changwen. 2010. Genetic variation in different populations of *Octopus variabilis* in China coastal waters based on the 16S rRNA gene analysis. Journal of Zhejiang Ocean University (Natural Science), 29(4):325-330.

-Morales, M.A. 2011. Infection or rather allergy? Foodborne Pathog. Dis. 8,749.

-Navas, A. and Albar, J. P. 2004. Application of proteomics to studies on phylogeny and evolution. *Proteomic* 4, 299-302.

-Navas, A. Cobas, G. Talavera, M. López, J.A. Ayala, J. and Martínez, J.L. 2007. Experimental validation of Haldane's hipótesis on the role of infection as an evolutionary force for Metazoans. *PNAS USA* 34, 13728-13731.

-Navas, A. López, J.A., Espárrago, G., Camafeita, E. And Albar, J.P. 2002. Protein variability in *Meloidogyne spp*. (Namatoda: Meloidogynidae) revealed by two-dimensional gel electrophoresis and mass spectrometry. *Journal of Proteome Research* 1, 421-427.

-Olsen, M.M., Heinecke, R. D., Skjødt, K., Rasmussen, K. J., Kania, P., Buchmann, K. (2011). Cellular and humoral factors involved in the response of rainbow trout gills to *Ichthyophthirius multifiliis* infections: Molecular and immunohistochemical studies. Fish and Shellfish Immunology 30, 859-869.

-Otero J, Salgado XA, González AF, Gilcoto M & Guerra A. 2009. High-frequency coastal upwelling events influence *Octopus vulgaris* larval dynamics on the NW Iberian shelf. *Marine Ecology-Progress Series*, 386: 123-132. -González AF, Otero J, Guerra A. & Pierce GJ. 2010. Age, growth and mortality of *Loligo vulgaris* wild planktonic paralarvae in the Ría de Vigo (NE Atlantic Ocean). *ICES Journal of Marine Science*, 67: 1119-1127.

-Pascual, S, González, A., Guerra, A. 2007. Parasites and cephalopod fisheries uncertainty: towards a waterfall understanding. *Review of Fish Biology and Fisheries*17:139-144

-Pascual, S., Abollo, E. 2008.Myxosporean infection in frozen blocks of Patagonian hakes. *Journal of Food Protection*. 71 (11):2316-2323

-Pascual, S., Antonio, J., Cabo, M.L., Piñeiro, C. 2010. *Anisakis* survival in refrigerated fish products under CO<sub>2</sub> modifiedatmosphere. *Food Control*, 21:1254-1256

-Pascual, S., González, A., Guerra, A. 2007.Parasite Recruitment and Oceanographic Regime: a working hypothesis on a global scale. *Biological Reviews* 82: 257-263

-Pierce, G.J., Caldas, M., Cedeira, J., Santos, M.B., Llavona, A., Covelo, P., Martinez, G., Torres, J., Sacau, M. & López, A., 2010. Trends in cetacean sightings along the Galician coast, north-western Spain, 2003–2007, and inferences about cetacean habitat preferences. Journal of the Marine Biological Association of the United Kingdom **90**, 1547-1560.

-Pierce, G.J., Santos, M.B., Murphy, S., Learmonth, J.A., Zuur, A.F., Rogan, E., Bustamante, P., Caurant, F., Lahaye, V., Ridoux, V., Zegers, B.N., Mets, A., Addink, M., Smeenk, C., Jauniaux, T., Law, R.J., Dabin, W., López, A., Alonso Farré, J.M., González, A.F., Guerra, A., García-Hartmann, M., Reid, R.J., Moffat, C.F., Lockyer, C. & Boon, J.P., 2008.

Bioaccumulation of persistent organic pollutants in female common dolphins (*Delphinus delphis*) and harbour porpoises (*Phocoena phocoena*) from western European seas: geographical trends, causal factors and effects on reproduction and mortality. *Environmental Pollution***153**, 401-415.

-Quiazon, K.M.A., T. Yoshinaga, and K. Ogawa. 2010. Experimental challenge of *Anisakis simplex* (sensu stricto) and *Anisakis pegreffii* (Nematoda: Anisakidae) in rainbow trout and olive flounder. Parasitol. Int. 60, 126–131.

-Quiazon, K.M.A., T. Yoshinaga, and K. Ogawa. 2011. Distribution of *Anisakis* species larvae from fishes of the Japanese waters. Parasitol. Int. 60, 223–226.

-Quiazon, K.M.A., T. Yoshinaga, K. Ogawa and M.D. Santos. 2009. Identification of larval *Anisakis* spp.(Nematoda: Anisakidae) in Alaska pollock (*Theragra chalcogramma*) in northern Japan using morphological and molecular markers. J. Parasitol. 95, 1227–1232.

-Quiazon, K.M.A., T. Yoshinaga, K. Ogawa, and R. Yukami. 2008. Morphological differences between larvae and *in vitro*cultured adults of *Anisakis simplex* (sensu stricto) and *Anisakis pegreffii* (Nematoda: Anisakidae). Parasitol. Int. 57, 483– 489.

-Robert-Pillot, A., Copin S., Gay M., Malle P., Quilici M.L. 2010. Total and pathogenic Vibrio parahaemolyticus in shrimp: fast and reliable quantification by realtime PCR. International Journal of Food Microbiology, 143, 190-197

-Rodríguez-Mahillo AI, González-Muñoz M., Moneo I, Solas MT, Mendizábal A, De las Heras C., Tejada M. Allergenic Properties and Cuticle Microstructure of *Anisakis simplex* L3 after Freezing and Pepsin Digestion. Journal of Food Protection, 71(12) 2578-2581, 2008.

-Rotariu, O., I. D. Ogden, L. MacRitchie, K. J. Forbes, P. Cross, A. P. Williams, C. J. Hunter, P. F. M. Teunis, and N. J. C. Strachan. In Press 2012. Applying risk assessment and spatial epidemiology to elucidate the source of human *E. coli* O157 infection. Epidemiology and Infection.

-Sánchez-Alonso I., Martinez I., Sánchez-Valencia J., and M. Careche Use of low Field NMR to estimate freezing storage time and quality changes in hake (*Merluccius merluccius*, L.) in 41st West European Fish Technologists Association (WEFTA) Annual Meeting, 27-30 September 2011, Gothenburg, Sweden.

-Sánchez-Alonso I., P. Carmona, M. Careche, Vibrational spectroscopic analysis of hake (Merluccius merluccius, L.) lipids during frozen storage. Food Chemistry In press. DOI: 10.1016/j.foodchem.2011.10.047

-Sánchez-Alonso I., I. Martinez, J. Sánchez-Valencia, and M. Careche, Estimation of freezing storage time and quality changes in hake (*Merluccius merluccius*, L.) by low field NMR, submitted to Food Chemistry.

-Santos, M.B., González-Quirós, R., Riveiro, I., Cabanas, J.M., Porteiro, C. & Pierce, G.J.In Press.Cycles, trends and residual variation in the Iberian sardine (*Sardina pilchardus*) recruitment series and their relationship with the environment. ICES Journal of Marine Science.

-Skov, J., Kania, Danish waters: A comparative study. Aquaculture 298, 24-28.

-Skovgaard, A., Bahlool, Q. Z. M., Munk, P., Berge, T., Buchmann, K. (2011). Infection of North Sea cod, *Gadus morhua* L., larvae with the parasitic nematode *Hysterothylacium aduncum* Rudolphi. J. Plancton Res. 33 (8), 1311-1316.

-Smith, J.M., Pierce, G.J., Zuur, A.F., Martins, H., Porteiro, F. & Rocha, F., 2011. Patterns of investment in reproductive and somatic tissues in the squid Loligo forbesii and Loligo vulgaris in Iberian and Azorean waters. Hydrobiologia **670**, 201-221.

-Strachan NJC, Doyle MP, Kasuga F, Rotariu O & Ogden ID (2005) Dose response modelling of *Escherichia coli* O157 incorporating data from foodborne and environmental outbreaks. *International Journal of Food Microbiology*, 103, 35-47.

-Strachan NJC, Dunn GM & Ogden ID (2002) Quantitative risk assessment of human infection from *Escherichia coli* O157 associated with recreational use of animal pasture. *International Journal of Food Microbiology* 75, 39-51.

-Tejada, M. Solas, M.T. Navas, A. Mendizábal, A. 2006. Scanning Electron Microscopy of Anisakis Larvae following Different Treatments. Journal of Food Protection 69, 1379-1387.

-Tejada, M., Solas, MT., Navas, A. and Mendizábal, A. Scanning Electron Microscopy of *Anisakis* Larvae following Different Treatments. Journal of Food Protection. 2006, 69(6): 1379–1387

-Thuy, D. T., Kania, P. W., Buchmann, K. (2010). Infection status of zoonotic trematode metacercariae in Sutchi Catfish *Pangasianodon hypopthalmus*: Associations with season, management and age. Aquaculture 302, 19-25.

-Traverso, A. 2011 A large outbreak of *Opisthorchis felineus* in Italy suggests that opisthorchiasis develops as a febrile eosinophilic syndrome with cholestasis rather than a hepatitis-like syndrome. Eur. J. Clin. Microbiol. Infect. Dis. 22,1-5.

-Vidaček, S. De las Heras C. and Tejada M. Quality of fish muscle infested with *Anisakis simplex*. Food Sc. Tech. Int.; 15(3):283-290, 2009a

-Vidaček, S. De las Heras C. Solas MT and Tejada M. Effect of high hydrostatic pressure on mortality of *Anisakis simplex* L3 and on muscle properties of infested hake. *J Sci Food Agric* 2009; **89**: 2228–2235 c

-Vidacek, S. De las Heras C. Solas MT, Mendizábal, A., Rodríguez-Mahillo AI, González-Muñoz M. and Tejada M. *Anisakis simplex* allergens remain active after conventional or microwave heating and pepsin treatments of frozen L3 larvae. JSFA. 89:1997-2002, 2009b

-Vidaček, S. De las Heras C. Solas MT, Mendizábal, A., Rodríguez-Mahillo AI, and Tejada M. Antigenicity and Viability of *Anisakis* larvae heated at different time-temperature conditions. J Food Prot 2010; 73(1): 62-68

Werner, M.T., Lin, A.H., Levsen, A., Egaas, E. 2011. A quantitative Sandwich ELISA for the detection of *Anisakis simplex* protein in seafood.*Eur. Food Res. Technol.* 232, 157-166.

-Wu Changwen, Chi Changfeng, He Guangyuan, Lv Zhenming, Xu Meiying. 2010. Isolation via enrichment and characterization of 10 polymorphic microsatellite loci in the cuttlefish, *Sepiella maindroni* de Rochebruns. Acta Oceanologica Sinica, 29(6): 121-124.

-Zuur, A.F. & Pierce, G.J., 2004. Common trends in Northeast Atlantic squid time series. *Journal of Sea Research* **52**, 57-72.