ParaFishControl

Advanced Tools and Research Strategies for Parasite Control in European farmed fish

Research and Innovation action: GA no. 634429

REPORT ON THE RISKS OF ZOONOTIC HELMINTHS FOR THE MAIN CULTURED FISH SPECIES IN THE EUROPEAN UNION AND PROPOSAL OF A FOOD SAFETY PROGRAMME

August, 2020

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TABLE OF CONTENTS

1 Executive Summary

This report is the result of five years of collaborative research within the **EU funded project ParaFishControl** (GA no. 634429). The project targeted, among others, the topic related to food safety identified in the SFS-10A call. This topic was included due to public health concern and economic importance of the fish-borne parasitic diseases in some European countries and also due to a previous EFSA report. The Panel on Biological Hazards of EFSA established a scientific opinion on risk assessment of parasites in fishery products (EFSA, 2010), that all wild caught seawater and freshwater fish must be considered at risk of containing any viable parasites (cestodes, trematodes, nematodes) of human health concern if these products are to be eaten raw or almost raw. The report also recommended studies to evaluate the effects of different farming practices on the prevalence of zoonotic parasites in aquaculture. One of the conclusions of this report was that Atlantic salmon, reared in floating cages and fed compound foodstuffs, are unlikely to contain live zoonotic parasites and therefore the risk of infection is negligible.

The Commission Regulation EU 1276/2011 stated that in the case that epidemiological data show that farmed fishery products are unlikely to contain live parasites under actual farming practices, they do not present a health hazard for fish-eating consumers. An exception was then included for farmed Atlantic salmon from the freezing treatment, which is mandatory for fish products intended to be consumed raw/undercooked.

However, the EFSA report also stated that, apart from farmed Atlantic salmon, sufficient monitoring data were not available for any other farmed fish and therefore it was not possible to identify which fish species do not present health hazard with respect to the presence of parasites. Therefore, along the PFC project we conducted a massive survey during several years, in which a total number of 10,587 samples from fresh water (rainbow trout and common carp) and marine (gilthead sea bream (GSB), European sea bass (ESB), turbot, Atlantic salmon (AS), marine rainbow trout) fish species were obtained from a representative number of fish farms in Spain, Italy, Greece, Denmark, Norway and Hungary. A random polietapic and stratified sampling plan was selected with a confidence level of 99%. In addition, primary processed products (fresh ESB and GSB) and secondary products (smoked AS), from supermarkets in France, Italy and Spain were also included in the study. The samples included also fish runts (fish discarded for commercialization) and fish farmed in vicinity to areas of high abundance of cetaceans. Identification of parasites was carried out with different methodologies depending on the type of sample (viscera, fillet, etc.) and fish species including visual inspection, UV-press method, artificial digestion, candling, muscular compression/artificial digestion followed by microscopic examination and PCR. Species identification of parasites was done by sequencing. No zoonotic helminths were found in any of the samples analysed (zero prevalence). A non-zoonotic helminth (*Holostephanus* sp.) was found frequently in common carp. Its zoonotic potential was discarded based on the literature and the negative results of the experimental infections run in rodents.

Although the examination of all the runts in the current survey has been negative for zoonotic helminths, we recommend to keep on discarding runts (as already done in many farms) from the processing line to the market.

Therefore, **we can establish that the overall risk of parasite infection in the selected farmed fish species is negligible and the pictured map of risks has an overall zero. According to these results, we propose to EFSA and European Commission to establish an updated scientific opinion and to consider the possibility to include more exceptions in the EU regulation** 1276/2011.

It may be concluded that there is strong evidence that EU farmed fish are reared in an environment that is free from viable zoonotic parasites. Clearly this evidence should be kept over time in such a way that food business operators verify through procedures that the evidence is still in force. Following the above reasoning, actions which unlock the value of systematize surveillance and diagnosis of zoonotic parasites across fish-farming practices and fish-value chains are helpful in monitoring risk, strength the competitiveness of the European Aquaculture and foster the consumer´s confidence. For doing so, we have also worked out **Good Practices Guidelines, an example of Certification Procedure with AENOR and Voluntary Control System (VCS)** that could be translated to the fish farming-marketing-retailer chain to monitor de absence of zoonotic helminths and to keep food safety.

The PFC consortium has disseminated these results to the scientific community (see section A7, the YouTube channel https://www.youtube.com/channel/UCldbO0L8Aya6NGIPpuc6uJQ; articles are in preparation or in press), and the Coordination has been in contact with several national associations of fish producers, the European Association of Fish Producers (FEAFP), the European Aquaculture Technology and Innovation Platform (EATiP, the Aquaculture Advisory Council (ACC) and several EU Policy Officers to translate these very important results to the Aquaculture Industry, **and now is time to go a step further into regulation.**

In the following pages, detailed information on the methods and results can be obtained. The report is divided in two sections: A: MAP OF ZOONOTIC RISKS FOR THE MAIN CULTURED FISH SPECIES IN EU, and B: ELABORATION OF A FOOD SAFETY PROGRAMME. This information is part of the know-how of the consortium and in particular of the partners involved in WP7: AZTI (A member of Basque Research and Technology Alliance), CSIC (Agencia Estatal Consejo Superior de Investigaciones Científicas), UNIBO (University of Bologna), KU (Copenhagen University), HCMR (Hellenic Centre of Marine Research), MTA (Hungarian Academy of Sciences) and UiB (University of Bergen).

A. MAP OF ZOONOTIC RISKS FOR THE MAIN CULTURED FISH SPECIES IN EU

1 Introduction

There is an extensive range of helminthic parasites of fish, but only a moderate number of species are capable of producing foodborne diseases in humans [1]. These diseases are either caused by an infection following ingestion of viable parasites, or by an allergic reaction against parasite antigens which occurs, so far, due to nematodes of the family Anisakidae. *Anisakis simplex* is the species most frequently associated with human disease, followed by *Pseudoterranova decipiens* [2] and *Contracaecum osculatum* [3]. In fact, *A. simplex* is the second most predominant biological hazard, constituting 33% of the reported hazards in 2009 [4]. This increase can be explained for several reasons: (i) the development of new and improved clinical diagnosis tests, (ii) the increase in raw and undercooked fish consumption and (iii) the growth in international market in fish products. Other fish helminths of zoonotic concern are cestodes and trematodes (especially Diphyllobothriids and Opisthorchioidea, respectively). They have received less attention despite of the reported human cases in Europe caused by these freshwater fish-borne zoonotic parasites Thus far, the available epidemiological data for farmed freshwater fish are scarce and necessary. For these main reasons in 2010, EFSA recommended studies to evaluate the effects of different farming practices on the prevalence of parasites in aquaculture [5]. As a matter of fact, one of the conclusions of this report suggests that Atlantic salmon (AS), reared in floating cages and fed compound foodstuffs, are unlikely to contain live zoonotic parasites and therefore the risk of infection is negligible. However, a later survey detected anisakid and raphidascarid larvae (*A. simplex* and *Hysterothylacium aduncum* respectively) in AS runts [6] which contrasts with a number of previous studies that confirmed no anisakids larvae in sea-farmed salmonids [5, 7-9]. These discrepancies were solved in a very recent study were 4,184 farmed AS, including runts, were sampled and examined in 2014/15. The fish were collected from 37 different salmon farms and confirmed that the presence of zoonotic parasites was restricted to runts and occurred in a very reduced number of runts (3/657); suggesting that the risk of any parasitic nematodes to occur in the flesh of farmed Norwegian salmon intended for human consumption is very low [10]. In addition, a qualitative risk assessment analysis, developed in 2016, remarked that attending to the current knowledge of the biology of the system, and the practices adopted in the AS farming, the overall risk of commercialization of product infested by viable larvae appears to be very low [11].

Another conclusion of EFSA report stated that, apart from farmed AS, sufficient monitoring data are not available for any other farmed fish and therefore it is not possible to identify which fish species do not present health hazard with respect to the presence of parasites [5]. The only work that shed light on this matter is a survey focused on farmed European sea bass (ESB), turbot (TB) and gilthead sea bream (GSB) bred in Spanish farms. This survey remarked that, similarly to AS, the presence of viable larvae appears to be indeed very low and therefore these species do not present a significant risk due to the presence of zoonotic parasites [12].

Concluding, it is generally assumed that farmed fish products have a very low or null prevalence of these helminths. However, this assumption has not been demonstrated scientifically and even less globally for the main European farmed fish species. The objective of the current work is to clarify the potential sources/routes of infection and design management strategies to decrease the occurrence of zoonotic helminths in farms and to obtain safe, high-quality fish food products following the basic principles and application guidelines for HACCP Hazard Analysis and Critical Control Point (HACCP). HACCP was developed to control the safety of processed foods and subsequently is practiced in a closed environment of a food factory [13]. The development of an HACCP plan for an aquaculture facility requires the examination of the production system and breaks down the whole process into stages to produce a flow chart of the process and the establishment of map of zoonotic risk.

2 Material and Methods

2.1 Farmed fish sampling

From spring 2016 to spring 2017 a total of 9,187 farmed fish have been examined from the following species: GSB, ESB, TB, AS (including smoked fillets), Marine Rainbow Trout (MRBT), Rainbow Trout (RBT) and Common Carp (CC); obtained from a representative number of fish farms located in Spain, Italy, Greece, Denmark, Norway and Hungary. A random polietapic and stratified sampling plan was selected with a confidence level of 99%. In addition, primary processed products (fresh ESB and GSB from Greece, Croatia and Turkey) from supermarkets in Italy and Spain were also included in the study. As the first round resulted to be negative for zoonotic helminths in all the species and locations, a second round in 2018 was focused in i) sampling fish that are normally discarded for commercialization (runts), ii) in sampling farms close to marine environments with abundance of cetaceans (involved in the life cycle of the zoonotic helminths) and iii) common carp farms with incidence of other helminths. In the second round, a total of 1,400 GSB, ESBand RBT fish runts were collected from Spanish, Danish, and Italian farms in 2018. Similarly, the second survey carried out in Italy examined further 260 GSB and 260 ESB runts from a farm where a specimen of ESB had been found positive for a larva of *Hysterothylacium fabri* during the first round survey. Moreover, 260 GSB and 260 ESB runts were also examined from a farm located in a sea area, the Ligurian Sea, where the presence of Pelagos Sanctuary and a high number of cetaceans, definitive hosts for Anisakid nematodes, could greatly influence the presence of these zoonotic helminths in ESB and GSB farmed in this area. In Spain 65 ESB and 65 GSB runts were also examined in two farms located in Alicante and Burriana in the Mediterranean area. 90 CC from one the Hungarian fish farms (North-eastern area) were sampled. Finally, 130 runts and 140 harvest quality RBT were collected from an Italian farm where heteroxenous parasites had been evidenced during the first round survey and 260 RBT were collected from a farm located in a

catchment basin endemic for Diphyllobothriasis in Northern Italy. Fish samples are summarized in the Table 1.

Table 1. Number of collected samples for farmed fish, during two rounds of the survey. The number of runts from the second round appears in brackets for each species and country.

AS	60	-	-	-	-	-	270[1]	330
TB	-	-	1035	-	-	-	-	1035
ESB	-	1571 [520]	65 [65]	1125	-	-	290[2]	3051 [585]
GSB	-	1563 [520]	65 [65]	1125	-	-	352[2]	3105 [585]
CC	-	-	-	-	1122	-	-	1122 [90]
RBT	-	1594 [130]	-	-	-	150	-	1744 [130]
MRBT	-	-	-	-	-	200 [100]	-	200 [100]
Total	**60**	**4728 [1170]**	**1165 [130]**	**2250**	**1122**	**350 [100]**	**912**	**10587 [1400]**

[1] Smoked Atlantic salmon sampling in local supermarkets (see 3.2 section)
[2] Whole GSB and ESB imported from Greece, Turkey and Croatia have been sampled from Italian and Spanish markets

2.2 Smoked Atlantic salmon sampling

The sampling was carried out temporarily (spring "P1" and autumn "P2") in local supermarkets in the Basque region (Spain and France) and Italy during 2016. Since the expected prevalence of anisakid larvae should have been low, we established a sample size of 270 salmon fillets between 100-200g, and the confidence level was set at 90% with an acceptable margin of error set at 5%. Sub samples size was split per suppliers, reflecting their AS commercial production volume in 2015 in the countries under study (Table 2). In addition, from spring 2016 to spring 2017, 13 samples of smoked fillets of wild sockeye salmon, 2 from supermarkets in Spain and 11 from Italy, were also collected as control samples.

Table 2. Sampling plan for smoked AS.

Producer		Estimated GWE tonnes	Estimate annual production (%)	Supermarket	Number of samples			Country
					Total	**P1**	**P2**	
Marine Harvest	Morpol	80,000	30	ESSELUNGA	51	14	37	Italy
	Harvest							
	Freihofer (Laschinger Morpol)			Aldi	8	4	4	Spain
Labeyrie	Labeyrie	30,000	10	COOP	43	43	0	Italy
	Delpierre			Intermarché	13	7	6	France
Ubago		15,000	5	Mercadona	20	9	4	Spain
Norvelita		15,000	5	COOP	15	0	15	Italy
Suempol (Norfisk Berlin)		15,000	5	Lidl	13	7	6	Spain
Mer Alliance		15,000	5	Carrefour	14	7	7	France
Delpeyrat		15,000	5	Carrefour	14	7	7	France
Xantelmar		7,500	3	El Corte Inglés	9	5	4	Spain
Starlaks		7,500	3	ESSELUNGA	14	0	14	Italy
Salmon Sur		7,500	3	Eroski	8	4	4	Spain
La Balinesa		7,500	3	El Corte Inglés	8	4	4	Spain
Koral		7,500	3	Eroski	8	4	4	Spain
Intermarché (Odyssee)		7,500	3	Intermarché	8	4	4	France
Fjord		7,500	3	COOP	12	12	0	Italy
Ahumados Dominguez		7,500	3	Opencor	8	4	4	Spain
El Duende		<5,000	1	El Corte Inglés	4	2	2	Spain

GWE: Gut weight eviscerated

2.3 Parasite inspection procedure

Identification of parasites was carried out with different methodologies depending on the type of sample (viscera, fillet, etc.) and fish species including visual inspection, UV-press method, artificial digestion, candling, muscular compression/artificial digestion followed by microscopic examination and PCR. Species identification of parasites were done by sequencing. The different procedures are detailed as follows:

Immediately after sampling, smoked fillets AS and whole fish samples were placed in a cooler and transported to the laboratory where they were kept refrigerated and then processed. Each whole fish sample was eviscerated and filleted and every fillet was placed separately into a clear plastic bag ensuring there is enough space in the bag for compression. Viscera were stored frozen until processing. Using a suitable system, squeeze fillets, including smoked AS samples, until they are approximately 1-2mm thick in a hydraulic pressing device at 7-8 bar. Then fillets, including smoked AS samples, were frozen at -20ºC for at least 48 hours and examined under ultraviolet light (365 nm), where anisakid larvae, if present, would appear as a fluorescent body [13]. Fillets were previously subjected to visual inspection by candling, as provided by the current regulatory framework. Artificial digestion of viscera has been performed by the peptic digestion method as defined in the Codex STAN 244, 2004.

Viscera were homogenized with sterile water and two independent DNA isolation was performed from 300 mg of the homogenate after mixing with 300µL extraction buffer (1% (w/v) SDS, 150mM NaCl, 2mM EDTA, Tris-HCl pH 8.0) supplemented with 40µL 5M guanidine thiocyanate, 50µL proteinase K (600 UmL-1) and subsequently incubated at 56 (±5) °C overnight. After centrifugation for 5 min at 16,000 g the supernatant was purified using Wizard Genomic DNA Purification Kit (Promega). DNA quality and quantity were determined with a NanoDrop Spectrophotometer (Thermo Fisher Scientific Inc.). PCR amplification of mitochondrial cytochrome c oxidase II gene (COII) was carried out following the protocol previously described in López and Pardo, 2010 [14].

With regard to identification of anisakid and raphidascarid larvae found during the survey in smoked fillets of AS and wild sockeye salmon, and whole ESB and GSB carried in Italy, larvae were isolated and identified at the genus level on the basis of morphological features and at the species level by PCR-RFLP of the ITS rDNA region, following the protocols reported by other authors [15-17].

Concerning RBT sampled from the Italian farms during first and second round surveys, fish were placed in a cooler then transported to the lab and immediately processed. Each fish was eviscerated, and visceral organs were subjected to visual inspection as provided by the Regulatory framework searching for larvae of Diphyllobothriid cestodes. All the sampled fish were filleted, and fillets subjected to visual inspection (including candling) by slicing the fillets in 2 mm thick slices. RBT from 3 farms were also subjected to search for metacercarial stages of Opisthorchioidea by compression and artificial digestion according to the protocol of the European Union Reference Laboratory for Parasites, Istituto Superiore di Sanità, Rome, followed by microscopical observation.

Common carps were examined by artificial digestion methodology as follows. The fish were sedated by adding a few drops of clove oil to their water and were killed by a cervical cut. After measuring of body weight and standard size, the whole musculature was removed. During the control, tiny muscle pieces were suppressed between two glass plates and the detected metacercariae were freed manually or by pepsine-digestion that contained 2 litre of tap-water, 10 g 1:10000 NF pepsin powder (Molar Chemicals, Halásztelek, Hungary) and 16 ml 25% HCl. These ingredients were mixed in a beaker, after which the solution was heated on a magnetic stirrer to 37°C. The fillets were immersed in the solution; after 20 min, the whole musculature had dissolved and intact metacercariae were collected following filtration after Erasmus 1962. After the elutriation of digestive solution hundreds of cysts could be collected with glass pipette and studied their morphology under a dissecting as well as a compound microscope. The most visible and characteristic body parameters (body length and width, size of pharynx, oral and ventral suckers, length of caecum) of 15 metacercariae were measured and documented in live condition (Erasmus, 1962). In addition, the fresh samples were photographed using an Olympus BH2 equipped with DP20 digital camera by 4X, 10X and 20X magnification.

In order to evaluate the zoonotic potential of the *Holostephanus* sp. found in CC, experimental infection trials were performed in rodents (mice, hamsters). Two mice and four Syrian hamsters were fed with 100 metacercariae of *Holostephanus* sp., while two mice and two hamsters were used as negative control. As a positive control, rodents were fed with zoonotic *Metagonimus* sp. metacercariae from common nase (*Chondrostoma nasus*) collected from the Danube river. The rodents were purchased from a commercial supplier (Ökomester Bt., Budapest, Hungary) and kept on a non-medicated chick starter diet. Formal ethical approvals were given by the Government Office of Pest County (permits No.: PEI/001/1792–4/2014 and PEI/001/1004-4/2015). Rodents were killed by CO_2 and their intestines were separated into three main parts (duodenum + jejunum, ileum and colon) and studied under a Zeiss stereo microscope for trematode infections (Fig.1).

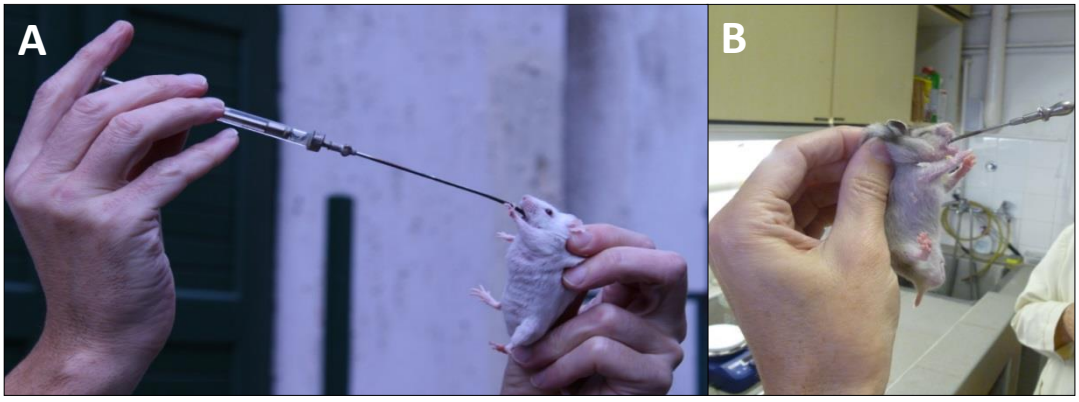

Figure 1. Forced infection of mouse (A) and Syrian hamster (B) with helminths.

Moreover, viability experiments of metacercariae from common carp were conducted during exposure to different physical treatments (temperatures of −18°C, +20°C, +40°C and +60°C) and chemical agents (5% and 10% acetic acid and 10% sodium chloride (NaCl)). Metacercariae lost viability by freezing at −18°C (2 h), heating at 60°C (20 min), incubation in 5% and 10% acetic acid (5 min) and 10% NaCl (2 h). These methods served as models to investigate the effectiveness of food preparation techniques (such as cold and hot smoking,

freezing, salting and pickling) on the survival of metacercariae. Control metacercariae (0.9%, +20°C) were able to stay alive for much longer (22 days).

3 Results

3.1 Survey of zoonotic parasites in marine fish species

No zoonotic parasites were found in any of the examined marine fish at the level of confidence of 99% with a margin of error of 4-8%. Only one L4 specimen of the raphidascarid nematode *H. fabri* encapsulated on the surface of the liver in one ESB from one Italian farm has been found.

3.2 Survey of zoonotic parasites in freshwater fish species

No zoonotic parasites were found in any of the examined freshwater fish at the level of confidence of 99% with a margin of error of 4-8%. However, we have found muscle samples harbouring metacercaria of *Holostephanus spp*. in CC with an overall prevalence of 10.64% (114/1122) from Hungarian fish farms. During the first year, 36 of 258 carp fingerlings (13.9%) were infected in the North-eastern farm. In addition, in the second year survey of the infected farm, heavy infection was found again, with prevalence ranging from 100% (30/30, in one-year-old) to 70% (21/30, in two-year-old) and 90% (27/30, in three-year-old carps). When the metacercariae from this CC were fed in an infection experiment to rodents (mice and hamsters), the trematodes were not able to develop in mammals. Therefore, it seems that *Holostephanus* species does not have any zoonotic potential based on both this negative results from experimental infections and literature review. As expected, the already known zoonotic helminth, the *Metagonimus* sp. used as positive control, were found in the intestines of rodents, 22 metacercariae and 5 adult *Metagonimus* were isolated from two hamsters. On the basis of the results on metacercariae viability, it seems convincing that culinary practice and preservation methods commonly used in Hungary and in most European countries can prevent the survival and possible transfer of metacercariae present in fish fillets.

3.3 Survey of zoonotic parasites in runts

No anisakids larvae were present in any of the ESB and GSB and RBT runts collected in Spanish, Danish and Italian farms.

3.4 Survey of zoonotic parasites in smoked AS samples

No anisakids larvae were present in any of the smoked farmed Atlantic salmon samples commercialized and intended for human consumption. Conversely, 10 (76.9%) out of 13 smoked fillets of wild sockeye salmons were positive for Anisakis larvae. Twenty-eight parasites (including also fragments referable to a single parasite) were found under UV light

examination; of these, two had not been detected by candling. Once those parasites were isolated from the fillets the morphological study allowed to identify all the larvae as *Anisakis sp*. type 1 (*sensu* Berland, 1961). Molecular identification by PCR-RFLP and comparison of the obtained sequences through BLASTN identified the nematodes as *A. simplex* (s.s.) with a 99% of similarity.

4 Conclusions

In conclusion, no zoonotic helminthic parasites were detected in marine and freshwater samples, even in runts, thus the prevalence is zero. Attending to the results obtained from different surveillances across Europe, besides the current work, we can establish that the overall risk of parasite infection in the selected farmed fish species is negligible (Table 3).

Table 3. Map of helminthic zoonotic parasite infection.

Fish species	Production system (*)	Adult feeding (*)	Susceptible for parasitic infection in wild environments (*)	Zoonotic parasites found [Monitoring data available]		Overall risk of parasite infection
				in harvest quality fish	in runts	
Atlantic salmon	Cages	Pellets	*A. simplex, P. decipiens, Metagonimus* spp.	None: [this work], [5, 7-10]	*A. simplex* [6, 10, 11]	Negligible
Gilthead sea bream	Cages/ponds	Pellets	*A. simplex, A. pegreffii, P. decipiens, Hysterothylacium* spp	None: [this work], [18]	None [this work]	Negligible
European sea bass	Cages/ponds	Pellets	*A. simplex, A. pegreffii, P. decipiens, Hysterothylacium* spp	None: [this work], [18] *A. pegreffii* [19]	None [this work]	Negligible
Turbot	In door tanks	Pellets	*A. simplex, P. decipiens*	None: [this work] [18]	Unknown	Negligible
Rainbow trout (**)	Cages/ponds	Pellets	*Diphyllobothriid, Opisthorchioidea, A. simplex*	None: [this work], [20,21]	None [this work]	Negligible
Common carp	Ponds	Pellets	*C. sinensis, O. felineus, M. takahashii, Haplochis taichui*	None: [this work], [22]	Unknown	Negligible

(*) source EFSA Report [5]
(**) including marine rainbow rout

However, some considerations should be considered for future investigations attending to available data:

- Fish feed could be a potential source of allergenic peptides from zoonotic fish parasites, since fishmeal obtained from marine pelagic fish is an important ingredient in aquafeeds used for the culture of Atlantic salmon and other fish species, and in the poultry industry. Thus, these feeds should be considered as a source of potentially allergenic peptides in the final products (fish fillet consumed by humans)[23] [24]. Although this risk is beyond the scope of this study, it is interesting to point that future studies are needed to evaluate if these parasite allergens can really pass from feeds to fillets and induce allergenic reactions in consumers, and if the potential can be decreased by the replacement of fish meal by other protein alternative sources.
- Although the examination of all the runts in the current survey has been negative for zoonotic helminths, we recommend to discard them (as already is done in many farms) from the processing line to the market, since these farmed fish are not properly fed and therefore, they could feed on anything that can be eaten in order to survive

including, invertebrates and/or “sneakers” in open cages that can infect them [6]. More samplings of runts should be done in future surveys.

5 Partners involved in the work

AZTI, IIM-CSIC, UNIBO, HCMR, KU, MTA, UiB

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B. ELABORATION OF A FOOD SAFETY PROGRAMME

1 Introduction

Over the last few decades, the presence of zoonotic nematodes in fish products has become a great concern for stakeholders, administrations and consumers. In fact, although the first regulation related to the management of these parasites was issued by the Dutch Health Authorities almost 50 years ago, since then, sanitary/social alarms increased substantially, revealing a paradox by which zoonotic parasites in fish have been considered not only a classic hazard but also an emerging (or re-emerging) risk (D'Amico et al., 2014). From the 1960´s to 1990´s, National Authorities in Germany, French, Italy and Spain introduced recommendations and regulations (some of them voluntary) as preventative treatments to help control the parasite hazards, especially focused on the process by which the fish industry guarantees parasite-free fish lots and to inactivate parasites in infected lots held to be sold for human consumption. Often, the management actions following the news on *Anisakis* broadcasted by the communication media in several European countries, which sometimes provoked unnecessary social alarm ("*worm hysteria*") episodes.

More recently, Commission Decision No. 93/140/EEC and the new Regulations introduced by the Hygiene Package (see No. 853/2004; 2074/2005; 1020/2008; 1276/2011) led to a radical change in the system of parasite control in fishery products. First of all, by defining the general framework by which a fish lot is determined to be fit or unfit for human consumption. Secondly, by sharing the responsibility for control in the fish value chain from veterinary inspectors to in-house programs managed by industry up to the final retailer, for whom in some European countries (e.g., Spain, Italy) is mandatory to inform the final consumer how to manage fish to be consumed raw or undercooked at home.

During the last 20 years, the above changes have provoked serious economic problems in the EU fish production value chain (Llarena et al., 2015). In fact, evidence of failure of the system, which includes rejection by consumers of unaesthetic heavily-infected fish species, the increased awareness of the already well-known clinical manifestations of anisakiasis and related allergies (Nieuwenhuizen and Lopata, 2014; Bao et al., 2017), seizure of infected fish lots during border controls or routine veterinary official inspections, and the economic losses due to rejection of fish lots by industry as a consequence of Hazard Analysis Critical Control Point (HACCPs) procedures, has become a topic of discussion in the Working Group on the Implementation of the Food Hygiene Package hosted by the European Commission (DG Health and Food Safety; SANTE).

Furthermore, a positive aspect of the new Hygiene Package regulations was the introduction into the value chain of the Risk Analysis perspective for controlling seafood hazards. The approach based on risk was recognized by standard-setting international organizations (Codex FAO/WHO, WTO and EFSA, in the context of seafood security, seafood market and

seafood safety, respectively) as being promising in risk control of the parasite hazards, by considering the entire sequential process (risk assessment, management, and communication) from net to plate. This holistic perspective made it possible to connect the advice derived from the scientific evidence (assessment) with the decision systems oriented towards solutions in the fish industry (management), and finally to transparently communicate verified risk information to end-users and seafood consumers. The risk analysis thus represented a declared guarantee that EU food safety regulations would be improved based on available knowledge, and that the control process in the industry would follow scientific criteria.

In 2010, the European Authority published a Scientific Opinion on risk assessment for parasites in fishery products provided (EFSA, 2010). The Opinion widely recognized that risk management was currently limited by many biological, ecological, medical and technological knowledge gaps. In 2012, the Commission launched the first time, a specific call on the topic "Food safety and quality issues related to parasites in seafood" (FP7-KBBE.2012.2.4-02) to offer a funding scheme for a collaborative project that addressed three key performance issues: the surveillance and monitoring of fish species/areas, the diagnostic awareness of allergic reactions, and the interventions in the food web to inactivate parasites. This resulted in the PARASITE (Parasite Risk Assessment with Integrated Tools in EU-fish production value chains) project (GA-312068), the most ambitious risk assessment plan to date. Dissemination of Project results had a positive impact on the fish sector, the administrations and the fish industry in general. The most significant milestone was the inclusion of zoonotic anisakids as etiological agents in National Plans for the Food Chain control, and in the European Rapid Alert System for Food and Feed System (RASFF).

Fortunately, the PFC Project gave way to enlarge in the short term the ongoing research on parasite risk assessment in wild stocks to marine and freshwater aquaculture systems. Based on a previous most ambitious surveillance plan ever conducted (Tasks 7.1 and 7.2), it may be concluded that the current risk of parasitic zoonotic infection for farmed marine and freshwater fish fed on compound feedstuffs is negligible (see D7.1).

Concerning the Atlantic salmon, reared in floating cages and fed compound foodstuffs, the risk of any parasitic nematodes to occur in the flesh of farmed Norwegian salmon intended for human consumption is also very low, occurring in a very reduced number of runts (Levsen and Maage, 2016).

With regard to freshwater fishery products, the epidemiological data available in literature for farmed freshwater fish suggest a negligible risk of zoonotic parasites as well. In fact, plerocercoid larvae of the zoonotic cestode *Diphyllobothrium latum* and metacercariae of zoonotic Opisthorchiid trematodes have not been described in freshwater fish farmed in Europe.

In PFC Project a large number of runts from marine and freshwater farms was examined, focusing on the productive systems in which heteroxenous not zoonotic parasites with a life cycle strictly linked to natural trophic webs, similarly to anisakid nematodes and diphyllobothriid cestodes (Fig. 1), had been detected during a first round survey.

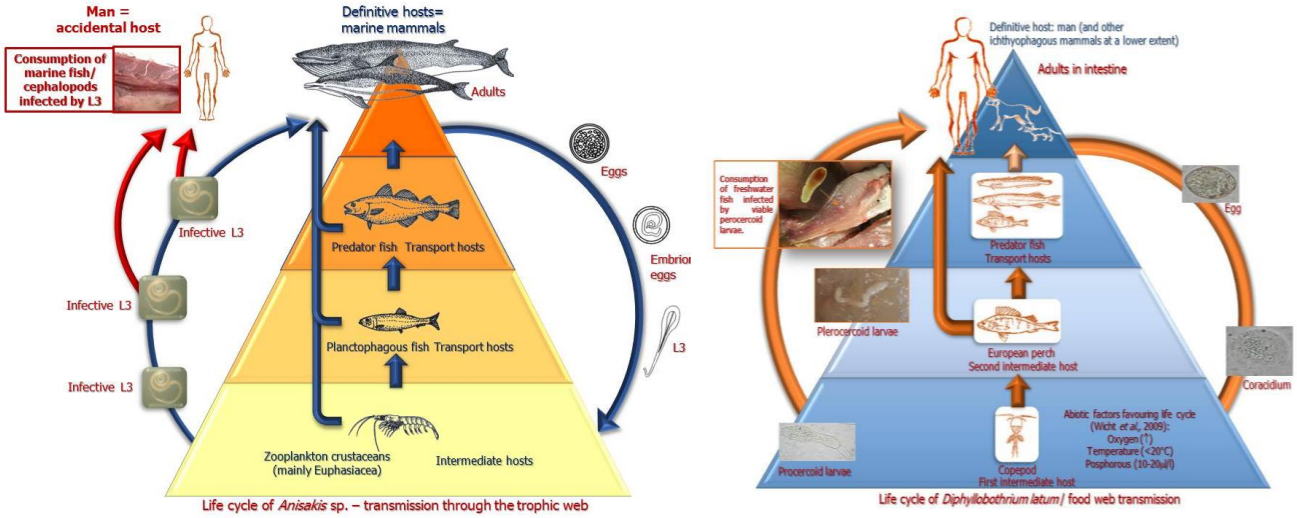

Figure 1: Life cycles of *Anisakis* spp. (left) and *Diphyllobothrium latum,* strictly linked to natural trophic web.

No zoonotic parasites were detected in runts, similarly to harvest quality fish examined during the whole survey. Overall, we can generally assume that, for the main European farmed fish products, a very low (“negligible”) risk of zoonotic parasites is expected when requirements and criteria stated in current regulations are respected, taking also into consideration the directions given by the “Guidance on viable parasites in fishery products that may represent a risk to the health of the consumer”, as schematized in Fig. 2.

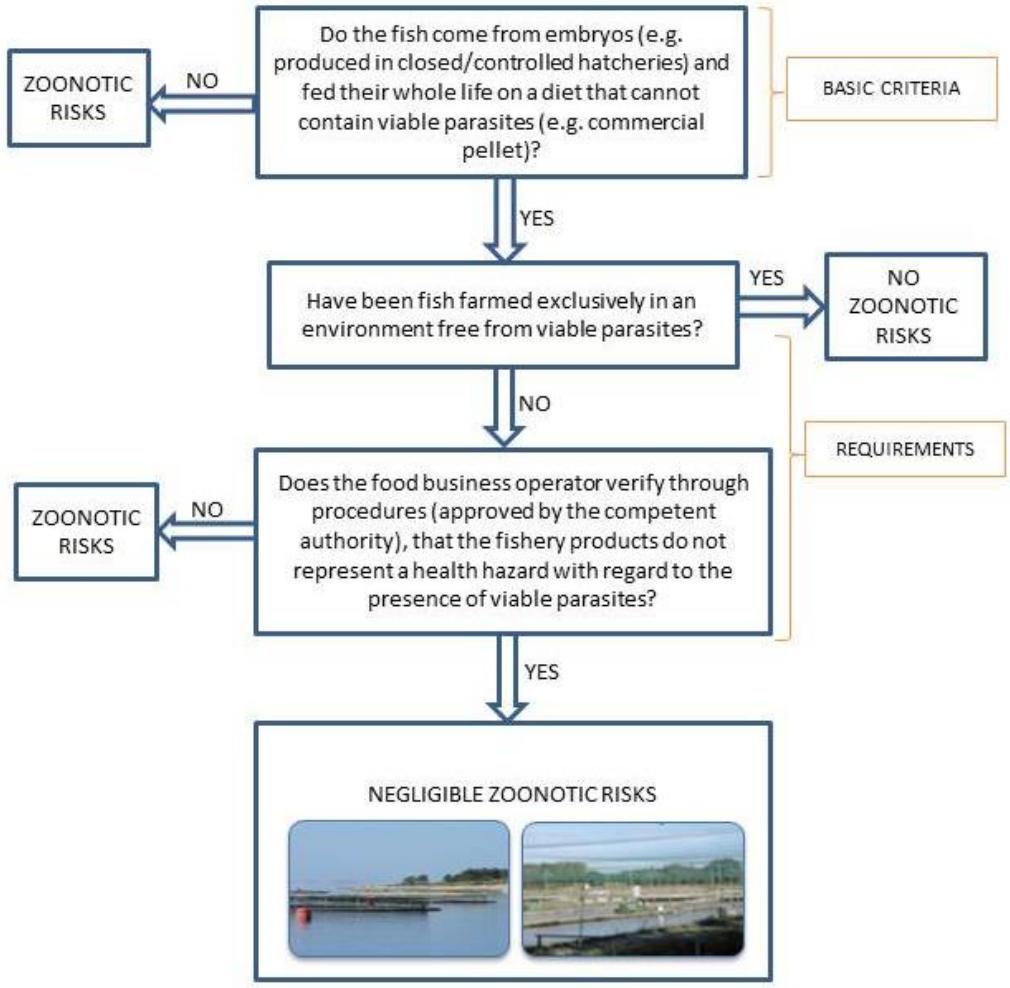

Figure 2: Flow chart of the basic criteria and requirements to be considered on the basis of EU legislation and official documents in order to evaluate the zoonotic risk due to parasites along the production chain

However, under a HACCP perspective the assumption of a “zero technical risk” must not only be proven, but monitored. First, monitoring is essential to food safety management in that it facilitates tracking of the operation. If monitoring indicates that there is a trend towards loss of control, then action can be taken to bring the process back into control before a deviation from a critical limit occurs. Second, monitoring is used to determine when there is loss of control and a deviation occurs at a Critical Control Point. Third, it provides written documentation for use in verification.

2 Good practice Guidelines

An approach adopted consensually among the PFC partners was to provide on the fingers of one hand a very simple practical indication on how to integrate what we have learned in PFC into a general HACCP plan with a double-aim: first, promote more transparent scientific information on risk assessment for zoonotic parasites in Aquaculture production; then, the dissemination of effective management actions (linked to efficient risk communication strategies) to guarantee the control of likelihood of hazardous parasites occurring in foodstuffs of aquaculture origin. We have looked at the recommended actions along the fish-

production value chain, distinguishing between pre- and post-harvest scenarios. We have also listed and described at the end (see Technical Annexes) some of the methods which may be intended to assist the industry.

2.1 Pre-Harvest (farmers)

2.1.1. Implement monitoring plans: routine epidemiological surveillance for all fish species/origins/production systems is a pro-active action to early detect potential (re)-emergent hazardous parasites and to early design the corrective measurements to be shared with rapid alert systems. These monitoring plans should include two procedural obligations: statistical confidence in sampling design and quantify uncertainty in analytical measurement. Monitoring epi-data should be managed within a traceable system (e.g., Biobank Platform; see PFC deliverable D6.4) as:

- Self-designed within a HACCP plan by a particular fish operator. A correct application of a Qualitative Risk Assessment system as internal self-control assessment of critical points linked to the zoonotic risk, already considered in previous analysis for Atlantic salmon (Crotta et al., 2016), would allow to make very unlike the chance of zoonotic parasites occurrence in all farmed fish.

- Outsourced by the operator to a specialized service (Academia, Public institutions, certified private companies).

- Integrated in a sectoral coverage under a Certification (e.g., the Spanish Specific Regulations on Conformity Certification for production of Aquaculture Fishery Products exclusively reared in an environment free of viable *Anisakis*) (see Annex IV).

- Integrated under a sectorial coverage as a Voluntary Control System (VCS). The main risk aspects to be monitored within the VCS are linked to a proper management of the fish farm, primary focused on the implementation of correct feeding protocols and appropriate management of the fish environment (see Annex V).

As an example, in this regard it should in fact be pointed out that, within the farmed fish population, the runts or “losers fish” generally represent specimens at risk of infestation with *Anisakis* or other nematodes with a similar life cycle (e.g. *Hysterothylacium* spp.) not being able to compete for food with the other bigger specimens (harvest quality fish) and so pushed towards the predation of potentially parasitized invertebrates and/or “sneakers” in open cages that may have entered the cage. Similar effect could originate by sub-optimal feeding protocols and management choices leading the “hangry” fish to move on the natural trophic chain. Additionally, all the precautions aimed to avoid the onset of the complex life cycle of the zoonotic parasites, such as covering the cages and the tanks to reduce the presence of birds (infected faecal material) or preventing the use of water source contaminated by faeces of suitable definitive hosts of zoonotic parasites should also be implemented.

Therefore, these findings lay the groundwork to the necessity of planning proper surveillance activities aimed at identifying the risk factors involved in the transmission of zoonotic helminths to farmed fish, and to identify any critical points to be monitored, providing solutions for developing good practice protocols and voluntary control systems to be applied

on each farm. A long-lasting application of this voluntary control system should guarantee an economic return to farmers in terms of better market price for fish products with a high safety level and a progressive optimization of surveillance sampling plans with a lower number of fish to be internally examined.

2.2 Post-Harvest (manufacturers, retailers)

2.2.1. Implement a new trustable inspection scheme

Risk management of zoonotic parasites should configure and consistently implement actions to ensure that scientific evidence is translated into action, while also considering aspects such as the key general principles established in EU food law (necessity, proportionality, minimum effect on competence) that guarantee and protect the functioning of markets. The development of the UV-Press and artificial digestion methods have been demonstrated useful in PFC (see Annex I). Both methods, **UV-press and pepsin-digestion, form an integral part of a new International Standard for the industry in the near future** (inspection method ISO 23036, *in preparation*). In particular, the UV-press has been proved as more accurate inspection scheme overpassing the current ambiguity of the visual inspection scheme, while meeting the compliance of scientific evidence with regulations. Gómez-Morales et al. (2018) evaluated the potential transferability of the UV-press method to the industry by a collaborative study involving industrial partners (β-testing). The UV-P reached 95.5% of accuracy, 94.4% of sensitivity, and 100 % of specificity, so the implementation of this method at the aquaculture industry is a challenge and an opportunity for the future.

2.2.2. Develop new technological approaches for hazard detection/diagnosis: It is of strategic market significance to explore new SMART solutions based on industrial technologies (e.g., artificial vision for entire parasites) and new -omics methodologies (parasite traces and their allergens) for the detection and diagnosis of hazardous parasites in matrices of fish. This task is linked not only with the goal of provide safer food, but also to enhance aquaculture industry competitiveness.

From previous results it is well-known that cultured fish must be fed with pelleted feed to guarantee that no viable zoonotic parasites enter the fish production value chain. Furthermore, regarding parasite allergens, more evidences in real conditions are still necessary to establish the incorporation from feeds to the farmed animals of potential allergenic parasite proteins. In fact, fish feed has been suggested as a potential source of allergenic peptides from the fish parasite. Recently, Faeste et al. (2015) performed an exploratory feeding trial using different feed types containing large amounts (20%) of processed larvae of *A. simplex*. In that work, the authors demonstrated the transfer of parasite protein traces with allergenic potential from the fish feed to the zebrafish tissue in a short period of time.

2.2.3. Explore the utility of risk integrating models

Risk-ranking techniques may be valuable, for instance in prioritisation when comparing relative risks from multiple seafood systems or from different intervention strategies. Risk ranking can be based on expert elicitations, qualitative measures or, more recently, developed on the basis of quantitative risk models. Risk ranking, using tools that rely on

knowledge of risk factors to rank risks and prioritize regulatory controls, is often used as a comparative risk scoring system that may be applied to any fish species/origin/production system.

Recently, Rodriguez et al. (2018) by analysing 2377 wild fish from 9 species and three important ICES fishing areas carried out a comparison of three different tools for determining the risk of *Anisakis* exposure in fish: i) a risk ranking tool registered as a trade mark (FPR standard) based on a previous risk categorization scheme, ii) the current visual method for fish inspection and iii) the UV-press/peptic digestion methods. The lowest rejection rate for each fish lot was found when using the FPR standard, which clearly suggest that a risk ranking tool represents a better trade-off between a best-value for money approach and the best-assurance for fish quality and safety approach than current inspection methods. Furthermore, if we fit the epi-data obtained in the PFC surveillance plan to the FPR standard, and then all fish lots would be labelled as excellent compared to many European wild fish stocks which are poor or fair regarding the parasite risk scoring.

Otherwise, Quantitative risk assessment (QRA) is a science-based methodology that estimates the probability and severity of an adverse event (e.g. health risk to individuals or populations due to exposure of zoonotic parasites through ingestion of contaminated wild fish meals). Used alongside Monte Carlo simulation methods, Bao et al. (2017) estimated the human health risk of anisakiasis simulating the uncertainty (lack of knowledge) and variability of the associated model parameters. The model integrate data obtained from social science methods (questionnaires and economic surveys) and from the natural sciences (fish parasite sampling surveys and infection rates in humans) in a process based QRA model. Despite the useful of QRA methods to test the effects of hypothetical seafood safety management scenarios (e.g., factors that can increase risk and interventions to decrease risk), to date no QRA model for zoonotic/allergic parasites in a farmed fish meal has been developed.

2.2.4. Improve Risk communication channels

Communication is of the greatest importance since implementation of this Food Safety Programme could not only produce healthier fish products but also lead to increased consumer and trade confidence in EU fish production systems. It is therefore important to develop tools to share risk information to become transparent and smarter (available, structured, ready for action). We must put in place tools (e.g., information technological intelligence reports, horizon scanning programmes, willingness to pay evaluations …), which capitalize the socio-economic implications of risk exposure at local, regional, national and European levels.

3 Conclusions

The EU Fish Aquaculture industry is one of the largest worldwide. Ensuring a safe and affordable food-chain, contributing to the sustainability of the blue economy and effectively managing food-borne hazards than can negatively impact on consumer perception, have been the challenges for the PFC project. This deliverable presents a practical guide on risk management for zoonotic parasites in farmed fish along the value chain. The five main subjects covered here (namely, the Implementation of a surveillance plan, the standardization of self-inspection schemes, the development and trial of new technological

approaches for the detection and inactivation of parasites and their allergens, the implementation of risk models and the improvement of risk communication) may set up the way ahead to many of the questions and open issues from stakeholders' perception on food safety in fish farming. Furthermore, these recommendations potentially benefit the end-users, both offering a real guarantee of a healthy fish consumption and by fitting the main consumer right of making a conscious decision based on transparent-scientifically evidenced choice.

4 Partners involved in the work

UNIBO, AZTI, IIM-CSIC

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6 Technical Annexes

ANNEX I - UV-PRESS

Scope: this method is applicable for the detection of Anisakidae L3 larvae (in particular to *Anisakis*, *Contracaecum* and *Pseudoterranova* genera) commonly found in marine and anadromous fishes. The method can be applied to any farmed fish, both for land-based fish farming and inshore-nearshore cage farms. This method allows quantifying parasitic infections by estimating the number of parasites in the fish musculature.

The method detects Anisakidae larvae in fish muscle tissue by UV examination after pressing and freezing. The UV-press method relies on the peculiar feature of frozen Anisakidae larvae, which show fluorescence under UV light, due to the presence of the pigment ("lipochrome") Lipofuscin. Nematode detection is based on screening under UV-light of flattened and frozen (–20 °C freezer) fillets, of either fresh or thawed fish. Samples are placed in clear plastic bags, pressed to a 1 mm to 2 mm thin layer (press-vacuum system, an automatic or manually operated hydraulic pressing device at 7 bar to 8 bar) and then frozen. After freezing and subsequent thawing of the fillets, visual inspection is carried out by examining each bag containing fillet under a 366 nm UV-light source. Any anisakid nematode larva will appear as a brightly fluorescent spot of different colours, partially depending on the anisakid species, so that it can be easily recorded and approximate site of infection may be determined.

Procedure: fresh or frozen fish shall be eviscerated and filleted, each flesh side/fillet put in separate (not necessary for small fish) transparent plastic bags. Proceed in the same way if the sample to be tested is lightly processed fish. Put each bag containing fillets in an automatic or manually operated hydraulic pressing device at 7 to 8 bar, and press for a holding time of at least 5 s until pressed samples reach 1 to 2 mm thickness. After pressing, the bags containing fillets are kept in a conventional freezer at -20 °C until fillet is frozen stiff throughout (usually 24 h). Before testing, samples should be completely thawed at 4 °C or higher not exceeding room temperature. The time between thawing and inspection shall not exceed 12 h at room temperature, otherwise Anisakidae larvae may lose their fluorescence. Thawing can be omitted if results are urgently needed, since fluorescence is also visible in frozen samples. Put the bags containing the thawed fish fillet under a 366 nm UV light source placed in a dark room. The set-up of the UV-light source should be equipped with both up- and down-light. A set-up comprising six fluorescent light tubes (e.g., Philips TL-D 18 W BLB), with two and four tubes yielding down- and up-light, respectively, is recommended. Other UV light sources can also be used (e.g., LEDs). Any anisakid larvae present in a given sample will appear as brightly fluorescent spots, partially depending on the actual anisakid species. Checking on both sides of the bag, the approximate infection site of the larvae can be recorded, i.e., whether they are in the dorsal (fillets) or ventral (belly flaps) portion of the fish flesh. Results shall be expressed as "present" or "absent" and number of Anisakidae larvae in "x" grams of sample, per fillet section. If requested or appropriate, the localization of larvae detected can be reported. If doubtful findings occur, confirmation and identification at the species level by molecular methods should be performed by a qualified reference laboratory.

ANNEX II - ARTIFICIAL DIGESTION

Marine Fish:

Scope: this method is applicable for the detection of Anisakidae L3 larvae (in particular to *Anisakis*, *Contracaecum* and *Pseudoterranova* genera) commonly found in marine and anadromous fishes. The method can be applied to any farmed fish, both for land-based fish farming and inshore-nearshore cage farms. This method allows quantifying parasitic infections by estimating the number of parasites in the fish musculature and, when applied to fresh fish or lightly processed fish products (never frozen before processing), determining the viability of Anisakidae L3, which may be present.

The artificial digestion method relies on enzymatic degradation of muscle fibres in a fluid composed of pepsin and hydrochloric acid followed by filtration and washing steps. The method detects Anisakidae larvae in fish muscle tissue allowing a differentiation between dead and viable anisakid larvae, if the temperature of the digestion solution does not exceed 37 °C (with the exception of *Hysterothylacium* sp. larvae, which are killed at 37 °C), and assuming the fish was never frozen.

Procedure: The minimum individual sample size for testing by digestion shall be at least 25 g and no more than 200 g. Fish shall be manually eviscerated, skinned and filleted, and the viscera and each flesh side/fillet can be put in separate transparent plastic bags to be analysed independently, depending on fillet size. To increase the surface area for enzymatic degradation, samples are gently eased apart taking care not to disrupt larvae by checking for them. Alternatively, a smasher/stomacher, that facilitates the digestion but does not damage the nematode larvae, can be used. Blending or grinding procedure should be avoided as they can damage or disrupt larvae. Anisakidae larvae are resistant to the pepsin (powder or granular: 1: 10.000 NF, 1: 12.500 BP, 2.000 FIP; liquid: 660 U/ml)-HCl 25% digest fluid and therefore can be recovered free from muscle tissues. To facilitate an efficient and rapid digestion, a maximum ratio of 1:20, meat to digest fluid, and a temperature of 37 °C ± 1 °C, shall be maintained throughout the process. The time required for digestion shall be 15 min to 30 min, but in case of muscle samples which are less digestible, the digestion time should be increased but, unless otherwise validated for a particular sample matrix, shall not exceed 45 min. Following digestion, the digest fluid shall be filtered through a sieve with specific mesh and retained larvae shall be rinsed with tap water. The digestion process is considered satisfactory if residual debris remaining on the sieve consists primarily of indigestible tissue of no greater than 5 % of the original sample mass. If undigested tissue remains on the sieve in excess, the digestion procedure shall be repeated. In the case of excessive undigested tissue, a new sample shall be collected and the entire method repeated. Anisakidae larvae shall be collected from the sieve and examined under the stereomicroscope with transmitted light at 10X - 20X magnification for their morphological identification or processed for further analyses. If positive or doubtful findings occur, confirmation and identification at the species level should be performed by a qualified reference laboratory, by means of morphological and/or molecular methods. The larvae can be transferred in a vial filled with 90 % ethanol and stored at a temperature range between -20 °C and 10 °C up to five years. Results shall be expressed as “present” or “absent” and number of Anisakidae larvae in “x” grams of sample, per fillet section. If requested or appropriate, the localization of larvae detected, as well as their viability, can be reported.

Freshwater fish:

Scope: this method is applicable for the detection of larval stages of zoonotic cestodes and digenean trematodes, namely plerocercoids and metacercariae belonging to Diphyllobothriidae and Opisthorchioidea respectively, in farmed and wild freshwater fish. The artificial digestion of the muscular tissue, previously subjected to muscular compression for metacercariae, can be employed to check the presence of plerocercoid larvae and trematode metacercariae in fish fillets. Similarly, to marine fish, artificial digestion allows to quantify parasite intensity by estimating the number of parasites in a standard amount of fish musculature. It is recommended to apply the method on fresh fish or lightly processed fish products. Frozen samples are not indicated because thawing of fish products could compromise the viability and the integrity of anatomical structures of parasites. Compression method is costless, without the use of expensive reagents, and gives the possibility to determine the exact location or infection site of metacercariae. Artificial digestion allows to process simultaneously a large number of samples and to isolate and have clean plerocercoid larvae and metacercariae for excellent morphology and easier identification.

Procedure:

Compression: during the filleting process, tiny muscle pieces from the head (cheeks, left and right) and from the base of fins (left and right) are pressed between two-glass slides, observed by stereomicroscope, then the detected metacercariae are manually freed.

Digestion: 50 g by side at least of musculature have to be subjected to artificial pepsin-hydrochloric digestion. Artificial digestion is prepared according to the Standard Operative Procedure of the European Union Reference Laboratory for Parasites (Istituto Superiore di Sanità, Rome, Italy - http://old.iss.it/binary/crlp/cont/SOP_Artificial_digestion_of_fish_fillet.pdf). In a baker containing 2 litres of tap-water, add 10 g 1:10000 NF pepsin powder and 10 ml of 25% HCl (molar concentration: 7.8-7.9). These ingredients are mixed and the solution is heated on a magnetic stirrer to 40 ± 2 °C. The muscular samples are chopped and immersed in the solution. After 20 min, or when the whole musculature is dissolved, the resultant liquid is filtered by 500 µm mesh sieves, facilitating the passage of intact smaller metacercariae in a sedimentation cone and retaining the bigger plerocercoid larvae. When the sediment is separated from the supernatant, through away the latter and wash with tap water the sediment. Repeat this step until the supernatant is clear. After the final sedimentation, pour 10 ml of sediment and physiological saline in a Petri dish and analyse it under the stereomicroscope at 15-20 magnification to detect metacercariae. Plerocercoid larvae of diphyllobothriid cestodes could be tentatively classified at genus level on the basis of some morphological feature (scolex shape and introflexion rate, tegumental ridges of the body surface and microscopical microtriches aspect).After morphology, plerocercoids can be transferred in a vial filled with 70% ethanol and/or dry stored at -20 °C temperature for their molecular identification, according to Wicht B., Yanagida, T., Scholz, T., Ito, A., Jiménez, J.A., Brabec, J. 2010. (Multiplex PCR for differential identification of broad tapeworms (Cestoda: Diphyllobothrium) infecting humans. J. Clin. Microbiol. 48/9, 3111–3116).

Concerning digenean larvae, it is important to stress that freshwater fish can harbour in their muscle tissues many species of metacercariae without zoonotic importance. Metacercariae can be identified at the genus level by their morphology but a great experience in the specific

field is requested. The most visible and characteristic body parameters (dimension/diameter of encysted metacercariae, body length and width of excysted metacercariae, size of pharynx, oral and ventral suckers, morphology of genital primordia when visible) of at least 15 metacercariae shall be measured and documented in live condition. After morphology, the metacercariae can be transferred in a vial filled with 70% ethanol and/or dry stored at -20 °C temperature for their molecular identification. A differential molecular diagnosis by multiplex PCR on Opisthorchioidea metacercariae of the most relevant zoonotic or potentially zoonotic species reported in EU countries, such as *Opisthorchis felineus*, *Pseudamphistomum truncatum*, *Metagonimus* sp., *Metorchis* sp. and *Apophallus* sp., has been set up in Deliverable 4.2 “Tools for detection and identification of zoonotic metacercariae and tests for quantitative monitoring of carp myxozoans in fish and the environments.

ANNEX III - REAL TIME PCR

Scope: This method is applicable for the detection of Anisakidae L3 larvae (in particular to *Anisakis*, *Contracaecum* and *Pseudoterranova* genera) commonly found in marine and anadromous fishes. The method can be applied to any farmed fish, both for land-based fish farming and inshore-nearshore cage farms. This method allows identify the presence of parasitic infections in the fish musculature, viscera and processed seafood products including ready to eat presentations and feeds. There is not an only procedure since a number of protocols have been previously published (see above). Two commercial kits are also available (PATHfinder Anisakis/Pseudoterranova/IAC 3-plex Assay, GENERON; Test kit for the qualitative detection of anisakid DNA by PCR Real Time, 4LAB Diagnostics). As an example, in the following section we detailed the procedure described in López and Pardo (2010).

Procedure: Twenty-five g of seafood sample is homogenized in presence of 25 mL sterile MilliQ H_2O and 40 µL proteinase K for 5 min. Some 300-400 mg of the mixture are mixed with 300 µL extraction buffer [1% (w/v) SDS, 150 mM NaCl, 2 mM EDTA, Tris-HCl pH 8.0] supplemented with 10 µL 5 M guanidine tiocianate, and 10 µL proteinase K and subsequently incubated at 56 °C for 1 h. After centrifugation for 5 min at 16.000 g the supernatant is purified using the Wizard DNA Clean-Up System (Promega) and finally eluted with 50 µl of sterile MilliQ H_2O. Final DNA concentration is measured by absorbance at 260 nm and stored at -20 ºC until used. DNA quality is estimated measuring the absorbance at 280 nm whereas the presence of undesirable RNA is evaluated on 0.6 % (w/v) agarose gels. Real Time PCR Amplification is performed in a MicroAmp Optical 96-well reaction plate. Amplification reactions are carried out with TaqMan™ Universal Master Mix (GE Healthcare). containing forward primer (5´-AGTAAGAAGATTGAATATCAGTTTGGTGA-3), reverse primer (5´-AAGTAAACTCAAAGAAGGCACCATC- 3´) and the specific TaqMan™ probe (5´-FAM-TTCCTACTTTAATTTTGGTTGCTC-MGB-3´) (22). Reaction is run on the ABI PrismTM 7000 sequence detection system, or similar with the following thermal conditions: 50 ºC for 2 min, 95 º C for 10 min followed by 40 cycles of 95 º C for 15 s and 60 º C for 1 min. We can conclude a positive result with a C_t value $\geq$ 39, corresponding to 40 ppm of parasite in 25 g of sample.

López, I. and M.A. Pardo, 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. Journal of Agricultural and Food Chemistry, 2010. 10(58(3)): p. 1469-1477.

Herrero, B., J.M. Vieites, and M. Espiñeira, Detection of anisakids in fish and seafood products by real-time PCR. Food Control, 2011. 22(6): p. 933-939.

Cavallero, S., et al., Validation of a commercial kit aimed to the detection of pathogenic anisakid nematodes in fish products. Int J Food Microbiol, 2017. 257: p. 75-79.

Godínez-González, C., et al., Quantitative SYBR Green qPCR technique for the detection of the nematode parasite *Anisakis* in commercial fish-derived food. International Journal of Food Microbiology, 2017.

Paoletti, M., et al., Species-specific Real Time-PCR primers/probe systems to identify fish parasites of the genera *Anisakis, Pseudoterranova* and *Hysterothylacium* (Nematoda: Ascaridoidea). Fisheries Research, 2017.

ANNEX IV - CERTIFICATION PROCEDURE (AENOR)

Contents:

1 Aim

This Spanish Specific Regulation describe, in compliance with section 3.2 of the General Regulations for Conformity Certificates of products and services (hereinafter the General Regulations), the specific system for certifying the production of AQUACULTURE FISHERY PRODUCTS EXCLUSIVELY REARED IN AN ENVIRONMENT FREE OF VIABLE *ANISAKIS*. The General Regulations prevail in all cases over these Specific Regulations. This certification is envisaged to be extended to other European countries.

The objectives of this certification process are to:

1) Guarantee that animals are reared exclusively in an environment free of viable *Anisakis*;
2) Ensure compliance of identification and traceability;
3) Ensure compliance with good handling practices for prevention of *Anisakis* contamination in processing/dressing rooms and sales points (in the case of non-packaged products);
4) Compliance with the analytical sampling plan.

2 Scope of application

This certification is used for fishery products that comply with all requisites of this document, applied to all steps in the supply chain:

- Fish farm;
- Processing rooms/dressing rooms;
- Sales points, in the case of non-packaged products.

3 Reference documents

Below are listed the references and full titles of the documents or rules that are cited in the rest of these Specific Regulations and which are valid at the moment this document was written.

It is the user's responsibility to verify the existence of more current applicable legal texts. They can henceforth be cited only using the respective reference (always without the year).

- General Regulations for Conformity Certificates (4th revision);
- European Regulation no. 852/2004 of the European Parliament and of the Council of 29 April 2004 concerning the hygiene of food products;
- European Regulation no. 853/2004 of the European Parliament and of the Council of 29 April 2004, establishing specific hygiene rules for food of animal origin;
- Regulation (EU) no. 1276/2011 OF THE COMMISSION of 8 December 2011, modifying annex III of Regulation 853/2004;
- European Regulation no. 178/2002 of the European Parliament and of the Council of 28 January 2002, establishing the principles and general requisites for food legislation,

creating the European Food Safety Authority and establishing procedures regarding food security;
- Regulation (EU) no. 1379/2013 of the European Parliament and of the Council of 11 December 2013, establishing the common organisation of markets in the sector of products from fisheries and agriculture;
- ANNEX D: Specific requisites of the system.

4 Definitions

Besides the definitions contained in the reference documents, the following definitions are considered:

APPLICANT COMPANY: Company applying for certification;

LICENSED COMPANY: Company to which AENOR INTERNACIONAL S.A.U. has granted the Certificate;

TRACEABILITY: the possibility of finding and following through all production, transformation and distribution phases the trail of a food, feed or animal meant to produce food or substance destined for inclusion in food or feed or likely to be so included;

TRACEABILITY SYSTEM (RE 178/2002): procedure which by means of registration, identification and transmission of information, enables products to be traced and located, from their production on, over the whole course of the commercialisation chain;

LARVAL REARING: Period comprised between egg hatching and larval growth up to inert feeding;

ALEVIN STAGE: Period from the start of inert feeding until completion of morphological development;

PRE-GROWING: Also named pre-fattening, intermediate period after the alevin stage, in which fish reach the JUVENILE STAGE and the right size and conditions for placement in growing facilities. This period and final fish size is very variable depending on the countries and farms;

GROWING: Period of fattening and growth of fish until reaching the commercial size;

FISHERY PRODUCT: All marine or freshwater animals (except live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods, as well as all mammals, reptiles and frogs), whether wild or farmed, including all edible products, parts and forms of those animals;

RGSEAA: (RD 191/2011) General Health Registry of Food Companies and Foods [*Registro General Sanitario de Empresas Alimentarias y Alimentos*]

REGA: (RD 479/2004) General Registry of Livestock Operations [*Registro general de explotaciones ganaderas*];

RESPONSIBLE OPERATOR/ORGANISATION: Operator designated by the organisation whose main functions are approval and monitoring of suppliers, handling complaints, conducting internal audits and managing nonconformities, etc;

COMPOUND FEED: Mixes of raw materials to feed animals, with or without additives, meant to feed animals orally in the form of complete or complementary feed;

HOMOGENEOUS PRODUCTION UNIT (HPU): Fish of the same species seeded in a REGA in the period of three months, from the same origin. The HPU will be considered a sampling batch.
For juveniles, each HPU corresponds to a different origin.

5 Management body

Management of this specific certification system is commissioned to the technical services of AENOR INTERNACIONAL S.A.U. (hereinafter AENOR), whose contact information is:

Address: Génova, 6 – 28004 MADRID – SPAIN
Telephone: (+34) 914 325 988
Email: agroalimentaria@aenor.com
www.aenor.com

6 Granting of the AENOR certificate

6.1 Granting process

The granting process shall conform to what is established in section 4 of the General Regulations and in the rest of this section.

6.2 Application

The company or, where appropriate, the legal representative interested in having the Conformity Certificate granted, shall submit the respective application on paper, with its own letterhead and in accordance with the content of the application form (Annex A), to the AENOR Technical Services.

That application should be accompanied by the questionnaire on general information about the company (Annex B). Relevant documentation on the food safety and quality system included in the scope of this certification should also be sent.

6.3 Initial visit

Once the application has been received, the AENOR technical services will prepare a control programme based on the information in the applicant's application. Dates will be scheduled for the different visits and it will be informed, by means of an audit plan, of the date and the respectively designated audit team.

For the initial assessment, the AENOR services, following AENOR procedures, shall apply the frequency of audits and analyses established in the following table:

TABLE 1: FREQUENCY OF AUDITS IN INITIAL VISIT – AQUACULTURE FARMS

TYPE OF AUDIT	NUMBER OF CENTRES TO VISIT	SUMMARY
INITIAL	An *in situ* visit to the aquaculture farms will initially be carried out to assess the implementation and effectiveness of the requisites set out in the applicable references. Samples will also be taken in the audited aquaculture farms; the volumes will be those established in Table 3.	AUDIT: 100% of AQUACULTURE FARMS if the number of farms is ≤ to 20. If the number of farms is > than 20, sampling of √n (rounding up to the whole number). SAMPLE TAKING: According to Table 3 (3.2 EXTERNAL MONITORING: Sampling plan for external monitoring) n: BY FARM TYPE
HALF-YEARLY UNANNOUNCED*: >20 sites	In the case of sampling for more than 20 sites: Later, between month 5 and month 7 since realisation of the initial certification audit another unannounced visit to the aquaculture farms will be made to ensure compliance with the set requisites, carrying out analytical sampling according to the criteria established in Table 3	AUDIT: If the number of farms is > a 20, sampling of √n will be done (rounding up to the whole number). SAMPLE TAKING: According to Table 3 (3.2 EXTERNAL MONITORING: Sampling Plan for external monitoring) n: BY FARM TYPE
UNANOUNCED AUDIT* (MIDWAY THROUGH THE CERTIFICATION CYCLE) ≤20 sites	If 100% of sites audited: Later, between month 16 and month 20 since the initial certification audit was conducted, another unannounced visit to the aquaculture farms will be made, to ensure compliance with the set requisites, carrying out analytical sampling according to the criteria established in Table 3.	AUDIT: 100% of AQUACULTURE FARMS if the number of farms is ≤ 20. SAMPLE TAKING: According to Table 3 (3.2 EXTERNAL MONITORING: Sampling plan for external monitoring) n: BY FARM TYPE

* The company will be notified (by telephone or email) of the date the unannounced audit will be conducted 48 hours before it is done. The notice will always be of an informative nature and that date cannot be changed unless because of duly justified force majeure.

TABLE 2: FREQUENCY OF AUDITS IN INITIAL VISIT: SALES POINTS, PROCESSING/DRESSING ROOMS

AUDIT TYPE	NUMBER OF CENTRES TO VISIT	SUMMARY
INITIAL	An *in situ visit* to the sales points and processing/dressing areas will initially be carried out to assess the implementation and effectiveness of the requisites set out in the applicable references. Samples will also be taken in the audited aquaculture farms; the volumes will be those established in Table 3.	AUDIT: A sampling of √n (rounding up to the whole number) of the processing areas and sales points will be done. SAMPLE TAKING: According to Table 3 (3.2 EXTERNAL MONITORING: Sampling plan for external monitoring)
HALF-YEARLY UNANNOUNCED *	Regarding the frequency of audits, the sampling will be that set for the aquaculture farms.	AUDIT: Sampling of √n (rounding up to the whole number) of the processing areas and sales points will be done. SAMPLE TAKING: According to Table 3 (3.2 EXTERNAL MONITORING: Sampling plan for external monitoring)

* The company will be notified (by telephone or email) of the date the unannounced audit will be conducted 48 hours before it is done. The notice will always be of an informative nature and that date cannot be modified unless because of duly justified force majeure.

TABLE 3. INTERNAL SELF-MONITORING/EXTERNAL SAMPLING PLAN

3.1 SELF-MONITORING: Sampling plan for internal self-monitoring

The sampling plan will be done according to standard ISO66020-1 “Sampling plans for batch inspections by batch tabulated according to the acceptable quality level”.

The sampling determined will be applicable for the initial audits and for the follow-up and for those half-yearly and midway through the certification cycle.

Noncompliance without justified cause of the established control frequencies will prevent the certificate from being renewed and/or maintained.

SAMPLING:

AQUACULTURE FARMS:		**SALES POINTS for non-packaged/bulk food**
ALL HPUs should be sampled. NOTE: The animals will be kept in quarantine until the analytical results are received.		Sampling of: 1 product batch/month.
GENERAL CRITERION* Samples/HPU	**INCREASE of analytical pressure by 25% over what is defined in the general criterion**	**CRITERION**
Sampling of the HPU will be done throughout the production process. Separate samples can therefore be sent throughout the batch's "life". In those batches where half of the batch is fished while the rest is destined for fattening, the method described above will be done in both stages/slaughters distanced in time. See specific requisites for more details about the sampling to be done in self-monitoring ** This is also applicable for mainland closed-circuit farms that divide batches depending on the animals' size.	1. In the case of frozen fish as food, in the food after the larval stage. 2. Use of non-extruded feed. 3. If *Anisakis* larvae are detected in a batch, this increase will be maintained during 3 consecutive samplings.	1 sample from the sampled batch should be taken.
The farms will send the internal self-control samples directly to the **AENOR LABORATORY**. The sample taking should be done at the product processing location. Samples should be in frozen state when sent.		

**** Specific sampling requisites in the self-monitoring**

1) INITIAL SAMPLING

The following will be taken:

- 13 fish = 13 samples in total between all HPUs with an average weight of more than 1000 g.
- 14 fish = 7 samples in total between all HPUs with an average weight of between 500 and 1000 g.
- 14 fish = 7 samples in total between all HPUs with an average weight of less than 500 g.
- Juveniles: 45 juveniles for each HPU will be taken (see definition of HPU for juveniles).

	No. OF FISH	No. OF SAMPLES
JUVENILES	45	3
FISH < 500 g	14	7
FISH (500 g – 1000 g)	14	7
FISH > 1 KG	13	13

The samples will be homogenised in the laboratory (if appropriate).

2) CRITERION FOR TRANSITION BETWEEN INITIAL TAKING AND FOLLOW-UPS

If the sum of that total of HPUs continues to evolve, the corresponding samples will be taken until reaching 13 samples; the sampling criterion will be the following:

- If the fish to be slaughtered weigh up to 1000 g: 6 samples will be taken = 12 fish;

- If the fish to be slaughtered weigh > 1000 g: 3 samples of fish (= 6 fish) with a weight of 500-1000 grams and 3 samples of fish (= 6 fish) with a weight > 1000g (= 6 fish) will be taken;

- In the event that the fish initially sampled > 1 Kg have continued more than 6 months since the initial control, 5 samples will be taken = 5 additional fish.

The samples will be homogenised in the laboratory (if appropriate).

3) SAMPLING IN THE FOLLOW-UPS:

The sampling criterion over the course of the batch's lifespan, FOR EACH HPU, shall be the following:

WEIGHT	**FEED FISH FARMS**	**FARMS FOR FISH WEIGHING ≤ 1000 g**	**FARMS FOR FISH WEIGHING > 1000 g**
0 – 500 g	13 samples = 26 fish	6 samples = 12 fish	4 samples = 8 fish
>500-1000g		7 samples = 14 fish	4 samples = 8 fish
>1000 g		0	5 samples = 5 fish

***If the initially sampled fish > 1 Kg continue more than 6 months since the initial control, then 5 samples will be taken = 5 additional fish.

	No. OF FISH	No. OF SAMPLES
JUVENILES	45	3

The samples will be homogenised in the laboratory (if appropriate).

*GENERAL CRITERION: The sampling plan described above applies to all types of aquaculture farms: mainland aquaculture, use of water disinfected with UV, use of filtered water, with entry of seawater or fresh/brackish water without filtering or disinfecting. It will also be applicable to cages in fresh, sea or brackish water.

Noncompliance without justified cause of the established control frequencies will prevent the certificate from being renewed and/or maintained.

3.2 EXTERNAL SAMPLING PLAN

AQUACULTURE FARMS:
Samples will be taken from a production batch/1 HPU per audited operation in accordance with what is described in the below table and according to the frequency set out in Table 1 of these Specific Regulations:

	No. of FISH	No. of SAMPLES
JUVENILES	90	6
ADULTS	13	13

The sample taking applicable to the aquaculture farms should be done at the product processing location, except when taking samples of juveniles.

SALES POINTS:
1 sample = 1 fish from 1 product batch should be taken.

The sample taking applicable to the sales points shall be done at the specific sales point sampled.

Clarification: At audited sales points, 1 product batch will be taken for analysis.

The samples will be sent directly to the **AENOR LABORATORY**. Sample taking should be done at the product processing location.

Sample taking will be done according to a three-part procedure: initial, verification and decisive. The samples for the decisive analysis will be held in custody of the company and should be kept frozen.

The verification samples will be held in the laboratory's custody and be at AENOR's disposal. They will be kept frozen. If the interested party does not agree with the results of the initial analysis, a verification analysis can be done. If the initial analysis and the verification analysis do not agree, a decisive analysis will be done at the AENOR laboratory.

Samples should be in a frozen state when they are sent.

Noncompliance without justified cause of the established control frequencies will prevent the certificate from being renewed and/or maintained.

6.4 Follow-up visit

Before the visit the certification's scope should be verified to determine whether there been any changes that entail readjustment of the specification conditions and/or the offer of certification.

The expected date for the different visits will be scheduled and the date and auditing team designated for that purpose will be communicated by means of an audit plan.

For the follow-up visit, the AENOR services, using AENOR procedures, shall apply the auditing and analysis frequencies established in the following table:

TABLE 4: FREQUENCY OF AUDITS IN FOLLOW-UP VISITS

The sampling plan will be the same one defined for the initial visit, regarding both external monitoring and internal self-monitoring.

AUDIT TYPE	NUMBER OF CENTRES TO VISIT	SUMMARY
INITIAL	An *in situ* visit to the aquaculture farms will initially be carried out to assess the implementation and effectiveness of the requisites set out in the applicable references. Samples will also be taken in the audited aquaculture farms; the volumes will be those established in Table 3.	AUDIT: 100% of AQUACULTURE FARMS if the number of farms is ≤ 20. If the number of farms is > a 20, sampling of √n (rounding up to the whole number) will be done. SAMPLE TAKING: According to Table 3 (3.2 EXTERNAL MONITORING: Sampling plan for external monitoring) n: BY FARM TYPE
HALF-YEARLY UNANNOUNCED*: >20 sites	If more than 20 sites sampled: Later, between month 5 and month 7 since the initial certification audit was conducted, another unannounced visit to the aquaculture farms will be made to guarantee compliance with the established requisites, carrying out analytical sampling according to the criteria established in Table 3.	AUDIT: If the no. of farms is > 20, sampling of √n (rounding up to the whole number) will be done. SAMPLE TAKING: According to Table 3 (3.2 EXTERNAL MONITORING: Sampling plan for external monitoring) n: BY FARM TYPE
UNANNOUNCED AUDIT* (MIDWAY THROUGH THE CERTIFICATION CYCLE) ≤20 sites	If 100% of the sites are audited: Later, between month 16 and month 20 since the initial certification audit was conducted, another unannounced visit to the aquaculture farms will be made to guarantee compliance with the set requisites, carrying out analytical sampling according to the criteria established in Table 3	AUDIT: 100% of AQUACULTURE FARMS if the number of farms is ≤ 20. SAMPLE TAKING: According to Table 3 (3.2 EXTERNAL MONITORING: Sampling plan for external monitoring) n: BY FARM TYPE

* The company will be notified (by telephone or email) of the date the unannounced audit will be conducted 48 hours before it is done. The notice will always be of an informative nature and that date cannot be changed unless because of duly justified force majeure.

TABLE 5: FREQUENCY OF AUDITS IN FOLLOW-UP VISIT: SALES POINTS, PROCESSING/DRESSING ROOMS

AUDIT TYPE	NUMBER OF CENTRES TO VISIT	SUMMARY
INITIAL	An *in situ* visit to the sales points and the processing and/or dressing rooms will initially be carried out to assess the implementation and effectiveness of the requisites set out in the applicable references. Samples will also be taken; the volumes will be those established in Table 3.	AUDIT: Sampling of √n (rounding up to the whole number) of the processing rooms and sales points will be done. SAMPLE TAKING: According to Table 3 (3.2 EXTERNAL MONITORING: Sampling plan for external monitoring)
HALF-YEARLY UNANNOUNCED*	Regarding the frequency of audits, the sampling will be than set for the aquaculture farms.	AUDIT: Sampling of √n (rounding up to the whole number) of the processing rooms and sales points will be done. SAMPLE TAKING: According to Table 3 (3.2 EXTERNAL MONITORING: Sampling plan for external monitoring)

6.5 Auditing process

The auditing process is meant to determine the conformity of the process associated to the certification of AQUACULTURE FISHERY PRODUCTS EXCLUSIVELY REARED IN AN ENVIRONMENT FREE OF VIABLE *ANISAKIS* and basically comprises:

- Visit to the aquaculture farms;
- Visit to the processing/dressing centres;
- Visit to sales points (in the case of non-packaged product);
- Analysis of records and documentation developed by the applicant to meet the system requisites established in Annex D.

The visit to the different centres subject to the scope should always coincide when there are some processes subject to the scope with the aim of carrying out the following actions:

a. *In situ* supervision of animals and products that are within the certification scope;
b. *In situ* supervision of production and/or handling and/or storage and/or distribution operations;

c. *In situ* supervision of expedition operations or, in the absence thereof if this action is not being done, documental supervision;
d. *In situ* supervision of records, documents and practices to verify compliance with what is established in ANNEX D;
e. Sample taking at the aquaculture farm/dressing room/sales point to verify the absence of *Anisakis* larva.

A documental review of the necessary procedures and record for compliance with the application requisites will be done per what is established in the reference documentation and in annexes C and D of these regulations.

It shall be verified that the processing of nonconforming products complies with the requisites set out in annex C of these regulations.

Once the visit has been made to each centre, whether initial or for follow-up or renewal, a report will be drawn up in duplicate and signed by the audit team and by the company's representative. A copy of that report will be left at the company or delivered later (within no more than 15 calendar days). If no information is available during the audit, the report will be sent once it has been received; the result of the audit will be conditioned by the study of that information.

6.6 Extraordinary visit

When noncompliance has been detected which might prevent the certificate from being granted (critical and/or major noncompliance), the AENOR Technical Services may decide to carry out an extraordinary visit to verify whether the detected nonconformities have been corrected.

6.7 Granting of the certificate

The Certificate of Conformity shall be granted when no major noncompliance (as defined in section 8) is detected or, where appropriate, if deviations (see section 8) are detected, *in situ* verification during an extraordinary visit will be conducted to ensure that the necessary corrective actions have been taken. That visit will not be necessary whenever it can be proved via documental control that the noncompliance has been resolved. The corrective actions should be reported within 30 calendar days after the visit.

For each audit carried out, the actions to correct noncompliance should be resolved within a maximum of 3 months from the date of the initial visit.

If that deadline is passed, the certification process will be cancelled and the certification application should begin again.

In the event of critical non-conformity, a complete new audit cycle will need to be carried out to proceed with granting the certificate.

7 Maintaining the certificate

7.1 Validity period and renewal

The maximum validity period of the AENOR Certificate will be three years. When that period is over the procedure indicated in section 6 of the General Regulations will be followed.

7.2 Follow-up audit

The follow-up activities will be adjusted to what is set out in section 5 of the General Regulations and in the rest of this section.

During the AENOR Certificate's validity period, the AENOR services will carry out the work indicated in the table of section 6.4, following what is indicated in the rest of that section, the aim being to verify that the conditions that led to the initial granting of the respective certificate have been maintained.

If appropriate, the audited centre will present a plan for corrective actions, following what is established in Section 6.7 of these Regulations.

7.3 Renewal audit

The AENOR technical services shall conduct a renewal audit every three years to verify that the conditions that led to the initial granting of the respective certificate have been maintained. If that audit is not done within the set time period then the certificate will be suspended.

The renewal audit will be done, at the latest, three months before the certificate's expiration date.

The work carried out in these activities covers the aspects reflected in section 6.3.

If appropriate, the audited centre will present a plan for corrective actions, following what is set out in Section 6.7 of these Regulations.

7.4 Expansions of scope

If situations where the licensee needs to expand the scope of the certification's management system occur:

- higher number of aquaculture farms means more sampling;
- substantial increase in density of animals due to increase of aquaculture farms or of their ability to remain within the certification scope;
- other.

This process should be audited by either a specific visit to evaluate the system associated to the new products or, if corresponding, by conducting a follow-up visit.

The work done in these activities covers the aspects indicated in section 6.3 regarding sampling level.

Based on the submitted information and in cases where there are no significant differences with the processes supervised at the last audit*, the AENOR Technical Services may process the proposal to modify the certificate's scope and this will be subject to special control at the next routing follow-up. In cases where there are relevant differences with respect to the last audit, an extraordinary audit may be conducted, as established in section 6.6 regarding verification of the system, so that the AENOR Technical Services can assess the situation.

*Up to 10% more new operations per year can be added to the list of producers associated to the certificate, without necessarily having to proceed with an AENOR control visit. When the number of operations increases by more than 10% per year, an AENOR inspection shall be requested previously, with a minimum sample of the square root of the total new operations.

8 Noncompliance, certification criteria and sanctions

8.1 Noncompliance classification for all operators

CRITICAL noncompliance is considered:

1. Critical noncompliance shall be considered the detection of more than 1 *Anisakis*-positive individual in the samples taken for each batch;
2. Systematic noncompliance in the identification of animals/products that entails or could entail noncompliance with the optional mention included in the certification scope, therefore supposing a risk to the programme's integrity;
3. Serious noncompliance regarding the tracing system which entails or could entail noncompliance with the optional mention included in the certification scope, therefore supposing a risk to the programme's integrity;
4. Absence of the pertinent authorisations as well as legal noncompliance;
5. Animals from non-approved aquaculture farms that do not comply with the established requisites, which entail or could entail noncompliance with the optional mention included in the certification scope, therefore supposing a risk to the programme's integrity (e.g. noncompliance with the established analytical control and quarantine, non-declaration of entry of animals or their origin and any other factor that could suppose a risk to the certification programme's integrity);
6. Systematic noncompliance in the management of non-conforming animals/products with respect to the specification conditions;
7. Noncompliance of the premises and actions determined for the analytical control plan with respect to the internal self-monitoring set out in these Specific Regulations (including the destination laboratory for the samples taken).

Critical noncompliance shall cause the certificate not to be granted or withdrawn. Its subsequent granting can only be accepted if an extraordinary audit is conducted which verifies the implementation, effectiveness and completion of the proposed corrective actions.

MAJOR noncompliance is considered:

1. Major noncompliance shall be considered the detection of 1 *Anisakis*-positive individual in the samples taken for each batch;

2. Noncompliance in the identification of animals/products that does not entail or might entail noncompliance of the optional mention included in the certification scope;
3. Noncompliance of some points of the tracing system which does not entail or might entail noncompliance of the optional mention included in the certification scope;
4. Occasional noncompliance in the management of non-conforming animals/products with respect to the specification conditions;
5. Animals from non-approved aquaculture farms but which can be shown to comply with the requisites in this specification. The noncompliance is due to management of the approval of suppliers, but does not or could not entail noncompliance of the optional mention included in the certification scope; the sampling plan set out in these Regulations was carried out according to the set criteria before bringing into the farm the batches of animals from that operator;
6. Systematic noncompliance with the requisites indicated in Annex D.

MINOR noncompliance is considered:

1. Any other occasional aspect detected concerning the aspects set out in ANNEX D and which would not be qualified as major noncompliance;
2. Merely documental aspects which do not suppose systematic noncompliance with any of the requisites determined in this specification.

8.2 Sanctions

NONCOMPLIANCE	MEASURE
CRITICAL	Temporary suspension or withdrawal of the certificate or non-granting in the case of initial audit. A complete extraordinary audit becomes necessary.
MAJOR	Request corrective action with evidence of implementation and completion, and verify in an extraordinary control that the problem was resolved (*). If not resolved, temporary suspension or withdrawal of the certificate or no granting in the case of initial audit.
MINOR	Request corrective action and verify that the problem was resolved at the next routine control.
Detection of *Anisakis* larvae	Withdrawal and recovery of the affected batch, as far as possible. Request corrective action with evidence of implementation and completion, and verify at the next extraordinary analytical control that the problem was resolved. Sampling intensity should be increased during 3 consecutive samplings, with a 25% increase in analytical pressure over what is determined in the general criterion of TABLE 3. In the case of the sales points: Sampling intensity should be increased during 3 consecutive samplings, the

	number of samples to be taken shall be 5 samples/batch. If same is not resolved, temporary suspension or withdrawal of the certificate or no granting in the case of initial audit.
** An extraordinary visit will not be necessary whenever it can be verified via documental control that the noncompliance was resolved.*	

During processing of instances of major noncompliance, as indicated in the General Regulations, AENOR may agree to temporary preventive suspension of the certificate.

In the event of preventive or temporary suspension:

Once the corrective actions proposed by the company have been implemented, an extraordinary visit will be conducted to verify their effectiveness. This assessment will be done before lifting the suspension.

9 Marking of certified products

The "AENOR certified" mention is the exclusive property of AENOR and can only be used by the licensed company based on the rules specified below:

- The mention may be directly associated to the **commercial documents** of batches corresponding to animals covered by the scope, must always be reproduced in that form so as not to mislead consumers and be associated to the certified characteristics of an "AQUACULTURE FISHERY PRODUCT EXCLUSIVELY REARED IN AN ENVIRONMENT FREE OF VIABLE *ANISAKIS*", entailing the following characteristics:

  i. Guarantees that the animals have been reared exclusively in an environment free of viable *Anisakis*;
  ii. Compliance of identification and traceability;
  iii. Compliance with good handling practices for the prevention of *Anisakis* contamination in processing/dressing rooms and sales points;
  iv. Compliance with the analytical sampling plan;

- Use of the "AENOR certified" mention is voluntary. However, if it is used, then compliance with the rules set out in this section is mandatory;
- Use of the optional mention in labels of the end product is always allowed as long as such labelling does not mislead consumers, the mention is associated to the certified characteristics of an "**AQUACULTURE FISHERY PRODUCT EXCLUSIVELY REARED IN AN ENVIRONMENT FREE OF VIABLE *ANISAKIS***" and the operator responsible for final packaging is within the certification scope and can demonstrate traceability to certified farms based on these Specific Regulations;
- In the case of sale at a fishmonger and/or in bulk, use of the optional mention in informative posters will be allowed as long as such labelling does not mislead consumers, the mention is associated to the certified characteristics of an "**AQUACULTURE FISHERY PRODUCT EXCLUSIVELY REARED IN AN ENVIRONMENT FREE OF VIABLE *ANISAKIS***" and the operator responsible for the sale is within the certification scope and can demonstrate traceability to certified farms based on these Specific Regulations.

The “AENOR certified” mention should in all cases be associated to the number of the certified organisation and to the scope and centres indicated in the corresponding Certificate.

The mention “REARED IN AN ENVIRONMENT FREE OF VIABLE *ANISAKIS*” is likewise authorised for use, as long as the premises described in these Specific Regulations are fulfilled.

No other mention other than those mentioned above can be used linked to the “AENOR certified” mention; these mentions cannot be used with other mentions not previously authorised by AENOR.

The licensed company must previously subject to the consideration of AENOR’s technical services all documents, physical supports and places where the “AENOR certified” mention will be used.

9.1 Abusive use of the “AENOR certified” mention

Use of the mention for the following will be considered abusive use:

- Products which are uncertified or were made at places other than those indicated in the contract;
- Products, processes or management systems which are uncertified or associated to facilities or centres other than those covered by the scope of the certificate;
- Products, processes, services or systems of management whose certificate has been temporarily suspended or definitively withdrawn;
- When its reproduction is not authorised by AENOR or is for purposes other than those authorised.

All abusive use of the “AENOR certified” mention or of the Certificate, whether by the applicant company, the licensee or a third party, gives AENOR the right to initiate, within the framework of current legislation, any judicial action it deems appropriate.

10 Applicable fees

AENOR shall establish and communicate to companies seeking certification the corresponding fees for activities associated to the granting, follow-up and renewal of the certificate. The fees will be indicated in the corresponding offer. Any change to the certification scope or to the expected work may imply a change to the offer initially presented.

Payments made during the granting process will in no case be reimbursed to the applicant company.

11 Appeals and claims

Action will be taken according to what is established in the General Regulations for Certificates of Conformity. For these Specific Regulations, the mandatory deadline for the initial response is 10 working days and 30 for the definitive resolution.

Annex A to the Specific Regulations for the Certification of Conformity for production of an AQUACULTURE FISHERY PRODUCT EXCLUSIVELY REARED IN AN ENVIRONMENT FREE OF VIABLE *ANISAKIS*

Mr/Ms ……, with National ID no. ……, in the name and on behalf of ……, domiciled at ……,

DECLARES

1 That it knows and pledges to abide by the General Regulations for Certification of Products and Services, the Specific Regulations on conformity certification for ……., and the commitments indicated therein;
2 That it pledges to pay the respectively corresponding costs, as established in the Specific Regulations;
3 That it pledges to unreservedly abide by the AENOR agreements concerning the processing of this application and the subsequent verifications and controls carried out as a consequence thereof;
4 That it pledges to guarantee that all operators commit to fulfilling the requisites associated to the certification scope;
5 That it pledges to provide to AENOR the list of suppliers and operations involved in the process and the references of products and/or animals applicable to the specification included in the organisation's system;
6 That it pledges to inform AENOR of any change regarding the system established in ANNEX D;
7 That it pledges to report any change in the scope;
8 That it complies with current legislation, especially that concerning working conditions and occupational health risks.

And therefore:

REQUESTS

That the AENOR Certificate of Conformity for the mention of "Aquaculture fish product exclusively reared in an environment free of viable *Anisakis*" be granted for the products indicated in the attached descriptive questionnaires, ……., reference ……, produced at ……..
…….

……, on the ……. of……., 20…..

SIGNATURE AND STAMP

Annex B

General Information Questionnaire

1 REGISTERED OFFICE OF THE COMPANY

1.1 COMPANY:
1.2 PLACE OF BUSINESS:
1.3 Telephone:
1.4 Fax: Email:
1.5 Corporate tax number (CIF):

1.6 CONTACT PERSONS

1.6.1 Person with sufficient legal capacity to sign the contract with AENOR
Mr/Ms
Position (of 1.6.1):
Tax number (NIF) (of 1.6.1): Telephone/fax (of 1.6.1):
Email (of 1.6.1):

1.6.2 Person for contact with AENOR:
Mr/Ms
Position:
Tax number (NIF): Telephone/fax:
Email:

2 AQUACULTURE FARMS (Should provide information about each centre whose inclusion in the certification scope is sought)

REGA: approximate % of production supplied:
Address:
Title-holder:
Contact person:
Telephone/fax: Email:
Farm type:
Average capacity:
Days when an unannounced visit is blocked (20 calendar days are permitted during which the certification body cannot visit the site(s)):

3 PRODUCTION INDUSTRY/PROCESSING CENTRE (if applicable) (should provide information about each centre whose inclusion in the certification scope is sought)

RGSEAA: approximate % of production supplied:
Address:
Title-holder:
Contact person:

Telephone/fax: Email:
Days when an unannounced visit is blocked (20 calendar days are permitted during which the certification body cannot visit the site(s)):

4 SALES POINT (should provide information about each centre whose inclusion in the certification scope is sought)

RGSEAA: approximate % of production supplied:
Address:
Title-holder:
Contact person:
Telephone/fax: Email:
Days when an unannounced visit is blocked (20 calendar days are permitted during which the certification body cannot visit the site(s)):

5 TYPES OF FEED/FOOD SUPPLIED

Name of farm	Supplier	Feed type/Food type

The truthfulness of the information contained in this questionnaire is the responsibility of the applicant.

……, on the ….. of ……., 20….

SIGNED:

(Name, position, signature and stamp)

6 OPERATOR/PRODUCT SUBJECT TO THE SCOPE

OPERATOR NAME	REGA / RGSEAA	PRODUCT SUBJECT TO THE SCOPE

The truthfulness of the information contained in this questionnaire is the responsibility of the applicant.

……, on the ….. of ……., 20….

SIGNED:

(Name, position, signature and stamp)

Annex C

Control system

1 Control of documentation

The different sites subject to the scope of this certification should determine and document a system for controlling documents and records associated to the requisites covered in these Regulations. The documents and records associated to the requisites required in these Regulations must be retained for a minimum period of 3 years (except for those documents with a longer retention period).

2 Requisites of the system for processing customer complaints

The company must maintain in place a customer complaint processing system regarding the certified system. The written procedure must describe at least the system for reception, registration, identification, analysis, follow-up and evaluation of customer complaints.

3 Processing of non-conforming products

A system for processing products that do not conform to these Regulations must be configured and documented. Records shall be maintained regarding the nature of those nonconformities, and of the corrective actions to take to eliminate the cause of real or potential nonconformities so as to prevent them from occurring again.

Nonconforming products should be processed taking into account the segregation of those products/animals that do not comply with the applicable requisites.

4 Product recall

The responsible operator will have a Product Recall Procedure to deal with potential problems. That procedure should contain at least the following information:

The identification of key personnel on the crisis management team, with clearly identified responsibilities.

- The criteria needed to decide whether it is necessary to withdraw a given product batch;
- An updated list of key contacts;
- A communication plan that includes providing timely information to customers and regulatory authorities;
- A plan that enables management of the logistics concerning tracing, recovery or elimination of the affected product and the review of existing stock.

The procedure will be tested at least annually. A maximum of four hours can pass from the time the crisis is activated until the actions to take are decided, including activation of the recall plan.

Annex D

Specific system requisites

1 General Requisites of the Specification's Control Management System

The general requisites the organisation responsible for the system established in these Regulations must comply with are set out below. The documents and records associated to the requisites required in these Regulations must be retained for a period of **at least 3 years**.

REQUISITE No.	GENERAL REQUISITES
1	The organisation must have a management system in place whose aims are to: 1) Guarantee that animals have been reared in an environment free of viable *Anisakis*; 2) Compliance of identification and traceability; 3) Compliance with good handling practices regarding the prevention of *Anisakis* contamination in processing/dressing rooms and sales points; 4) Compliance of the analytical sampling plan. Documentation control: the organisation must maintain in place a documentation control procedure that guarantees the conformity of operators according to the requisites of this specification.
2	The organisation shall maintain an updated list of approved operators which ensures proper control of same.
3	Internal audits must be conducted based on the following control frequency: CASE 1: no. of farms > 20, sampling of √n of audited farms: Annual internal audits of all operators will be conducted so that during the three-year certification cycle all operators associated to the specification have been subject to the set self-monitoring audits; if instances of noncompliance are detected, the necessary corrective actions will be implemented. CASE 2: no. of farms ≤ 20, 100% of audited farms: Annual internal audits of 100% of the operators associated to the specification will be conducted; if instances of noncompliance are detected, the necessary corrective actions will be implemented.
4	Control of all documents generated during the control of operators will be implemented. This will include, according to the method established, some of the following records: — Audit reports — Analytical reports — Records of management of nonconformities and derived corrective actions The operator responsible must conduct the determined self-monitoring regarding both the internal audits and the determined analytical control. There must be evidence of that self-monitoring.

5	Will be in charge of monitoring consignments/batches of animals associated to the specification.
6	Complaint management: the organisation must have in place a procedure for managing complaints. Special attention should be paid to complaints concerning the presence of *Anisakis*. **In the event of any complaint of this type, measures must be taken immediately, which may even lead to the withdrawal and recall of product batches as well as removal of batches in the production phase from the in-conformity list.** The certification body shall be informed of this circumstance as long as it has been noted that the complaint is due to the presence of *Anisakis* larvae in the fishery product.
7	A procedure for nonconformities and corrective actions shall be maintained, as well as all evidence needed to process and close the detected deviations for all nonconformities that may arise (self-monitoring/external monitoring/other).

2 Aquaculture farms

The documents and records associated to the requisites required in these Regulations must be retained for a period of **at least 3 years**. The information contained in them must also be truthful and up-to-date.

REQUISITE no.	REQUISITES OF THE AQUACULTURE FARM
1	The farm must be registered in the General Registry of Livestock Operations (REGA), as established in current legislation.
2	The farm must demonstrate its legal use of land and water and must have all licenses required by the corresponding autonomous community.
	ORIGIN OF THE ANIMALS
3	All animals included in the specification shall originate in approved aquaculture farms; compliance with the specification requisites shall be demonstrated at all times (animals reared in an environment free of viable *Anisakis*, correct identification, assured traceability, proper handling, segregation of product that does not conform to the specification, appropriate analytical plan according to these Regulations and with conforming results).
4	If juveniles or adults are obtained from non-approved aquaculture farms, a sampling must be taken according to the criteria set in Table 3 of these Specific Regulations before their introduction at the farm. The animals will be kept in quarantine until the results are received.
5	The sampling plan for internal self-monitoring must be fulfilled as specified in TABLE 3 (3.1).
6	An isolation facility must be maintained for introduced populations (quarantine) until reception of the analyses with conforming results.
7	The introduction of wild juveniles or adults shall not be permitted in the aquaculture farm unless pertinent measures for preventing cross-contamination with animals associated to the specification have been established; the evidence needed to guarantee this point must be available. In any case, those animals and the batches where they are introduced shall not form part of the batches of animals conforming to these Specific Regulations.
	TRACEABILITY
8	A system that ensure traceability must be established (which includes mass

	balance) for the eggs, larvae, juveniles and fish present at the farms.
OPERATIONAL RECORD AND IDENTIFICATION BOOK	
9	The operational record book should be filled out and up-to-date. The operational record book should contain at least the following information: •□Information on the title-holder •□Information on the operation •□Information on the farming •□Types of facilities •□Information on entries: Date, no. of examples, cause (purchase, birth, etc), REGA origin •□Information on exits: Date, no. of examples, cause (death, sale, slaughter, etc) •□Balance •□Official controls and inspections Each operation must keep updated its operational record book, where the necessary annotations will be made. The title-holder of the operation is primarily responsible in the event of noncompliance. The record book shall be available at the operation and must be accessible to the competent authority and/or for inspections by a third party, upon their request, during the period which they determine, which in any case cannot be less than three years. All movements of animals, both incoming and outgoing, must be supported by a shipment health form, which will be indicated in the operational record book.
10	The facility must maintain a record of sources and purchases of juveniles.
11	The identification system will enable the tracing and identification of the batch number the animals belong to at all times. The identification system should ensure that no animal not included in the certification scope can form part of the batch of conforming animals per the specification. Also, that system shall guarantee that the management of animals not conforming to the specification does not imply, during any of the production phases, a danger of *Anisakis* contamination for those animals that do conform.
12	Doubtful animals will always be identified so that they can be kept apart from suitable animals according to the specification of conditions, thereby assuring that they do not enter and form part of the batch of conforming animals with respect to the specification of conditions. It shall be guaranteed that those animals do not become a focus of contamination for conforming animals.
13	In the case of animal purchases, the animals must be accompanied by the form for the corresponding shipment and for their condition as suitable with respect to these Specific Regulations.
14	When they leave the operation the animals should be accompanied by the shipment form and the necessary documentation to guarantee the identification, traceability and declaration of conformity of the animal batches with respect to these Specific Regulations.
VETERINARY TREATMENTS/ANIMAL HEALTH VIGILANCE	
15	THE FARM MUST BE SUBJECT TO VETERINARY MONITORING. The farm must have an operational veterinarian or be associated to a livestock health protection group.
16	The facility must have a documented health management plan that is adjusted to the health status of the farm and to the group of positive findings for *Anisakis* presence detected over time.
17	The health management plan should contain a risk analysis that identifies ways pathogenic agents might be introduced into the rearing area or be transmitted to other facilities by its live aquatic products or waste (dead animals, faeces,

	food, etc).
18	The farm's practices should include regular vigilance of disease, sanitation and quarantine of sick animals.
19	The plan should explain how the set-aside or drying cycles used to interrupt parasite infection cycles are planned. Records of that planning should exist.
20	Treatments book: the treatments book should always be kept up-to-date, codified and properly paged. All treatments must be recorded, as well as the identification of those batches that have been treated.
21	The corresponding health qualification must always be available.
22	The legal withdrawal periods for all medicaments used must always be respected.
23	Medicaments not duly authorised by current legislation with respect to dosage and prescription shall not be used. Medicaments will always be prescribed by a veterinarian.
24	Veterinary prescriptions shall always be conserved for a period of not less than 5 years. The prescriptions will be codified and dated and include all required legal information.
25	The results of animal health monitoring must be recorded, particularly indicating any positive result that shows the presence of *Anisakis* larvae. Corrective actions taken to deal with such situation shall be indicated.
26	The follow-up procedures for indicators such as *Anisakis* presence in routine controls, complaints due to *Anisakis* presence, etc, should be described. The actions taken shall always be recorded.
27	The operation's health programme, whether individual or as part of a health protection group, should include control of the presence of anisakid nematodes. Evidence of the monitoring done and the measures taken, if necessary, must be retained.
	FEEDING OF ANIMALS
28	All feed destined for animals included in the certification scope shall come from authorised feed factories. Makers must comply with the legal requisites for the respective use to feed fish destined for human consumption.
29	As far as possible, except for larva cultivation, the farm shall use food based on extruded feed.
30	If fish is used as food in feeding after the larval stage, it shall always be frozen and the analytical pressure set out in the internal self-monitoring table shall be increased by 25%. The use of live feed or fresh fish shall not be permitted for the animals indicated in the specification. In the event that live feed or fresh fish is used for other animals not indicated in the specification, the food cannot at any time imply a focus of contamination for the conforming animals with respect to the specification conditions.
31	Feed should always be correctly identified; traceability for all purchases made shall be maintained.
32	If another food type is used (e.g. granulated feed), the analytical pressure shall be increased by 25% over what is set out in the internal self-monitoring table.
33	A system which guarantees the traceability of all feed received and applied in the aquaculture farm must exist.
34	Delivery documents shall include all information concerning the respective identification and traceability: date, destination, origin, feed type, batch and quantity.
35	Records of all feed used, respective sources and any test conducted to detect parasite presence must be kept.
36	Feed shall be stored in such a way as to prevent cross-contamination as well

	as respective deterioration; the appearance of pests shall also be prevented. Storage should always permit the proper respective inspection.
37	The formulations, in accordance with the legal requisites, for all feed used in the animals included in the specification conditions must be available.
38	Regarding medicinal feed and/or medicinal premixes, they shall be handled, stored and administered so that possible cross-contamination is always prevented.
39	All medicinal feed and/or medicinal premixes supplied shall be recorded, including date of administration and batch of treated animals.
40	Medicinal feed and/or medicinal premixes shall always be accompanied by their corresponding prescription.
BIO-SAFETY MEASURES AND CONTAMINATION CONTROL	
41	Rearing areas must control and monitor the origin and quality of the sources of water entering the animal rearing zones.
42	If cages are used, the farm must have a plan based on risk analysis to minimise *Anisakis* contamination from wild animals (including other fish).
43	If cages are used, documents indicating the measures taken to prevent piscivorous birds and marine mammals from entering the cages. Example: physical methods (nets, noise) to minimise bird presence, etc.
44	Facilities must manage physical interactions with wildlife. A wildlife interaction plan must exist, describing how to deal with and prevent the presence of piscivorous birds and marine mammals in the vicinity of the operation.
45	A list of piscivorous birds present in the operation's vicinity must be kept; the presence % must also be indicated.
46	SYSTEM FOR RECORDING MARINE MAMMAL SIGHTINGS: A system for recording sightings of marine mammals should be established. If a significant increase in their presence is detected, the cause of that increase should be studied.
47	Practices that foster the normal increase of the presence of marine mammals cannot be undertaken. The following practices shall be avoided: - Feeding of marine mammals by personnel of the farm; - If the facilities host tourist visits, controls must be in place to ensure that food is not given to marine mammals.
48	The facilities' design shall ensure that they are not favourable places for bird nesting or use as a refuge by any type of animal.
49	LOSS COLLECTION REPORT: If anisakid nematode larvae are found in a batch of fish, cannibalism may mean increased risk. A system for periodically collected losses should be implemented to minimise the risk of those losses being consumed by other animals in the batch. The collection of those losses must be recorded.
50	POSSESSION OF DOCUMENTS ATTESTING TO COLLECTION OF LOSSES BY AN AUTHORISED HANDLER FOR SUCH SUBPRODUCTS: Documentary evidence of proper management of mortalities through collection by a handler authorised by the respective authority for managing animal subproducts not destined for human consumption must be in place.
CLEANING AND DISINFECTION	
51	The cleaning and disinfection programme established by the company shall be fulfilled. There must be records of same.
52	Special attention should be paid to cleaning and, if appropriate, to disinfection of equipment and utensils that come into contact with the cultivation water and the cultivated species.
53	The cleaning and disinfection programme shall especially emphasise the prevention of contamination by *Anisakis* larvae due to improper management of losses, feed and waste, etc.

	TRANSPORTATION
54	The containers used to transport live animals must be clean and if reused must be cleaned and disinfected between uses. Records must be kept to guarantee this state.
55	The transportation of live animals shall always safeguard against the loss of traceability and identification of those animals conforming to the specification conditions.
56	The accompanying documentation should stay with the merchandise throughout the respective transportation.
57	In the event of transporting dead fish, the transportation shall be undertaken in such a way as to prevent cross-contamination and the migration of larvae between fish. GMPs shall include, among others: - Products conforming to the certification scope separated from those not covered; - Handling after hands washed; - Conforming products shall not be transported in containers that previously transported products not covered by the certification scope unless preventive measures have been taken to minimise possible cross-contamination; - Utensils that previously transported products not covered by the certification scope shall not be used unless preventive measures have been taken to minimise possible cross-contamination.
	INTERNAL SELF-MONITORING
58	A **documented procedure** should be in place, based on analysis of risk from *Anisakis.* Among others, that procedure should cover: traceability and identification, animal health monitoring, food control, GMPs, bio-safety, L&D and transportation, etc. There should be evidence of the actions taken.
59	The operator responsible for carrying out all necessary control activities must always be allowed to enter. The taking of samples determined in these Specific Regulations must likewise be allowed.
60	The premises and actions determined for the analytical sampling plan set out in the Specific Regulations should be fulfilled.
61	Appropriate measure shall be implemented in the event of any nonconformity that may arise. The necessary corrections and corrective actions shall be implemented as soon as possible.
	TRAINING
62	Fish should be handled by trained and qualified personnel. The companies should thus implement training plans that include correct practices for hygiene, handling, maintenance of health conditions, considerations of animal wellbeing and environmental preservation.
63	Anyone who employs or hires personnel charged with care should ensure that such personnel have duly received instructions and assessment in accordance with the certification scope.
	VISITS
64	Visitors should be provided with concise information about correct hygiene practices before visiting the facility.
65	Visitors are not allowed to come into contact with the water or with the fish.
66	Visitors shall not be allowed to feed the fish.

3 Sales points/processing-dressing rooms

The documentation and records associated to the requisites required in these Regulations must be retained for a period of **at least 3 years**. The information contained in them must also be truthful and kept up-to-date.

REQUISITE no.	REQUISITES AT SALES POINTS/PROCESSING-DRESSING ROOMS
1	The establishment shall have the corresponding authorisations.
GMPs: the aim is to minimise cross-contamination and migration of larvae in products	
2	During fish processing according to the specification conditions, a minimum separation from fish not included in the specification shall be maintained, always avoiding total or partial contact between both.
3	Before handling fish, hands shall be properly washed.
4	Containers, trays and equipment used to store fish must have been effectively washed before being used in the conforming product per the specification.
5	Utensils which previously transported products not covered by the certification scope shall not be used unless preventive measures have been taken to minimise possible cross-contamination.
6	If unpackaged fish are being transported, the transportation shall be done in such a way as to prevent cross-contamination and migration of larvae between fish. GMPs shall include, among others: - Conforming products with respect to the certification scope separated from those not covered; - Handling after hands washed; - Conforming products shall not be transported in containers previously used to transport products not covered by the certification scope unless preventive measures were taken to minimise possible cross-contamination; - Utensils which previously transported products not covered by the certification scope shall not be used unless preventive measures were taken to minimise possible cross-contamination.
7	Bulk fish sales point: fish conforming to the specification conditions shall maintain minimum separation from fish not included in the specification, so as to always avoid direct contact between both. Fish subject to the certification scope shall be situated 20 cm or more above fish not included in the certification scope.
IDENTIFICATION AND TRACEABILITY	
8	The traceability of the product included within the certification scope shall always be maintained.
9	Correct identification of the product included within the certification scope shall always be maintained. The identification shall include all the requisites detailed in these Specific Regulations.
INTERNAL SELF-MONITORING	
10	The premises and actions determined for the analytical sampling plan defined in these Specific Regulations must be fulfilled.
11	All appropriate measures shall be taken in the event of any nonconformity that may arise. The necessary corrections and corrective actions shall be implemented as soon as possible.
12	A **documented procedure** should be in place, based on analysis of *Anisakis* risk. Among others, that procedure should cover: L&D and GMPs, etc. Evidence of the actions taken must exist.

ANNEX V - VOLUNTARY CONTROL SYSTEM

Index

1. Introduction

The scientific opinion on risk assessment on parasites in fishery products established that farmed fishery products are susceptible of presenting zoonotic parasites. However, the epidemiological results obtained during last years, including the surveillance carried out in PFC project, reveals that the overall risk of parasite infection in farmed fish is negligible. In addition, Regulation EU 1276/2011 stated that food business operators need not carry out the freezing treatment of fish products consumed raw, marinated or salted derived from fish farming, cultured from embryos and have been fed exclusively on a diet that cannot contain viable parasites and therefore do not represent a health hazard. To guarantee that the overall risk of parasite infection in the farmed fish species is negligible and the pictured map of risks has an overall zero is necessary the implementation of actions to assure this fact, including monitoring plans, the compliance of identification and traceability and the compliance with good handling practices for prevention of parasites contamination. These actions are taking into account in a sectorial Voluntary Control System (VCS) in order to minimize the risk of the presence of viable parasites that could represent a health hazard. This document summarizes the objectives, structure, rules and guidelines of a sectorial VCS to strength the competitiveness of the European Aquaculture and increasing the consumer's confidence.

1.1 WHY VOLUNTARY CONTROL SYSTEM (VCS)?

- A reference standard for aquaculture is an important instrument, but cannot on its own guarantee food safety.
- A continuous monitoring of the industrial self-control organisations is needed to provide safe products.
- A European body to implement this industrial self-control concept and to harmonise rules, and guidelines can improve the effectiveness and efficiency.

1.2 OBJECTIVES

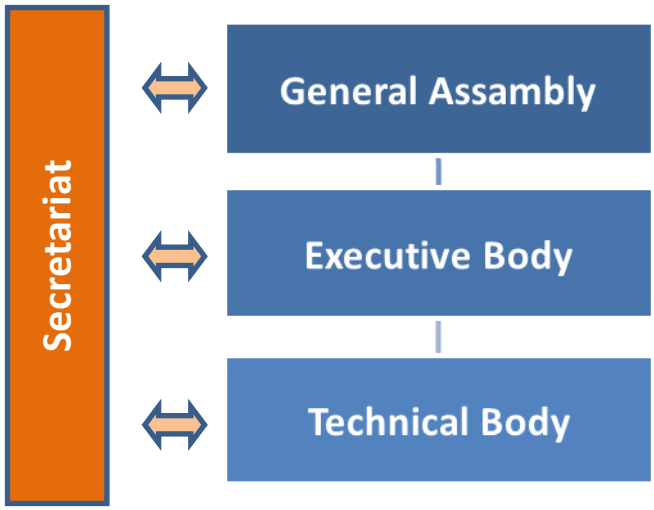
- To assure that the fishery products (derived from fish farming) do not represent a health hazard with regard to the presence of viable parasites.
- To assure the absence of zoonotic helminths in farmed seafood products.
- To protect the image of the products and the industry.
- To assure a fair competition in the sector.
- To develop control systems in countries where these not yet exist and to harmonise existing systems.
- To harmonise and to create consensus regarding the interpretation of analysis results on behalf of the members (the operational quality control systems).
- To create an early warning system for detection food safety issues.
- To provide an efficient raw material control.
- To be a platform for exchange of know-how and expertise.
- To provide a system of mutual information and communication of control results.

1.3 PRODUCT SCOPE

- The VCS scope should be defined within the system governance. It could be applicable for all chain from aquaculture farms to final distribution.
- This guideline is applicable for zoonotic parasites free environment for the following species: gilthead sea bream, European sea bass, turbot, rainbow trout, common carp and Atlantic salmon.
- This guideline could be used as a starting point in order to concrete and define a VCS for the aquaculture industry.

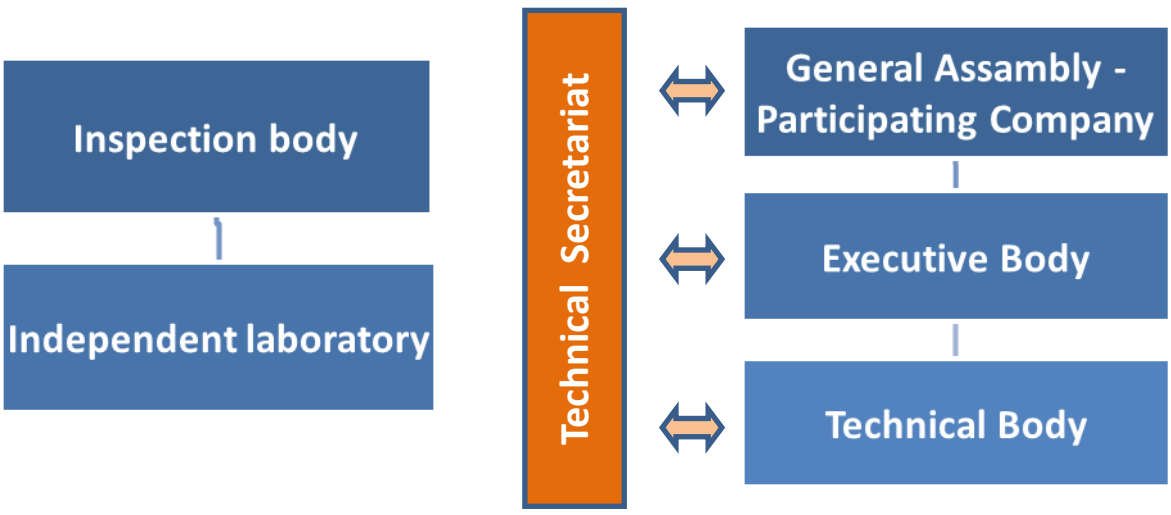
2. Structures and Rules

2.1 STRUCTURE



European Level (E-VCS)

National Level (N-VCS)



European level

The tasks and the composition of the European Aquaculture VCS-Bodies on European level could be described in its Statutes which must be approved by the General Assembly.

National/Regional level

The national/regional VCS are responsible for implementing the European Aquaculture VCS rules, guidelines and requirements on a national/ regional level.

Below different governance bodies are listed. Also tasks for each body and meeting proposed.

- **General Assembly**
  - ✓ **Tasks**
    - Appoint a Technical Secretariat.
    - Appoint the president and members of the executive body.
    - Promote the NVCS system.
    - Establish and control a central budget included membership fees.
    - Decide upon appeal of applicants to whom membership has been rejected by the executive body.
    - Establish office address for correspondence.
    - Responsible for implementation and execution of the EVCS guidelines at a national/regional level.
    - Approve plans and execute measures against companies that do not comply.

- **Executive Body**
  - ✓ **Tasks**
    - Supervise and implement the EVCS rules and guidelines on a national basis.
    - To maintain liaison and supervise independent laboratories and inspectors carrying out the EVCS tasks.
    - To send laboratory reports received, to the technical body for interpretation and comment.
    - Report on status of the EVCS at regular intervals to the supervising body.
    - Approval of independent laboratories, inspectors and experts.
    - Resolve problems relating to national disputes.
    - Approval of the sampling plan per participant/non participant and the related number of samples to be analysed according to the EVCS rules.
    - Track the actions and take decisions on external corrective actions.

- **Technical Body (group of experts)**
  - ✓ **Tasks**
    - Interpret and comment on the analytical results.
    - To recommend to the Executive Body actions in relation to technical and scientific matters.
    - Maintain liaison on all technical aspects of the EVCS through the National and European bodies.
    - Recommend for approval independent laboratories and inspectors.
    - To co-opt additional temporary members if special expertise is required and to seek financial support if necessary.

- **Technical Secretariat**
  - ✓ **Tasks**

    Proposes annual inspection, sampling plans and analyses.

    Manage the communications and actions derived from the evaluation carried out by the Technical Body based on the analytical results.

    Organize meetings for different governance bodies.

    Exercise responsibilities delegated by the Executive Body or General Assembly and represents the system with third parties (institutions, organizations or companies).

    Dissemination of the organization and its activities.

    Maintain contact with the European organization and participate in the derived activities.

- **Inspection Body**
  - ✓ **Tasks**

    Carry out inspections of participants according to relevant guidelines.

    Attend meetings of the National Executive Body and Technical Body as requested.
  - ✓ **Requirement**

    A background of aquaculture processing technology and analytical experience is necessary.

    Independent from individual companies of the sector and/or other sector associations.

    Experts must be approved by the Executive Body

- **Independent Laboratory**
  - ✓ **Tasks**

    Analyse samples as requested by the Executive Body according to the rules and guidelines.

    Report results of analysis to the NVCS /RQCS.

    To ensure all samples are correctly documented.

    Attend meetings of the Executive Body and Technical Body as requested.
  - ✓ **Requirements**

    Laboratory must be approved by the Executive Body and the General Assembly.

    Laboratory must be willing to participate in organised ring tests, which are carried out periodically upon request of the EVCS Board of Directors.

2.2 MEMBERS / PARTICIPANTS

European level

In the statutes eache type of member should defined, this below it is shown as an example:

1. Ordinary Members:
   1.1 Associations within the EEA and EU candidate countries.
2. Supporting members:

2.1 Individual companies and other legal entities willing to support the work and philosophy of the Association and provide financial sponsorship.

Application for membership will be made in writing. The Board of Directors will decide on membership at its next regular meeting. Should the Board of Directors reject an application, the applicant has the right to raise an objection at the next General Assembly. The decision of the General Assembly is final.

National/Regional level

Ordinary participants

Definition

Full owner of the goods.

Obligations

Accept all terms and conditions for ORDINARY participants according to the National Aquaculture VCS statutes.

Acknowledge responsibility in assuring compliance with food safety regulations (zoonotic helminths free environment).

Obliged to inform the NVCS / RQCS about encountered problems and when a problem arises to help solve the problem.

All members should define protocols and reference guidelines which should be used as a baseline for all VCS. AENOR reference could be an starting point.

Rights

Approved ORDINARY participants have the right:

To be represented as a Voting member in the "National General Assembly".

An optional right to obtain feed-backs on analyses and inspection-reports can be granted by a NVCS /RVCS.

To receive early warning information provided by the European VCS.

Supporting participants

Other companies, associations and/or other agents within the food chain, could share the same objectives, interest and goals of the VCS.

Definitions, obligations and rights of these supporting members are listed as an example.

Definition

Do not process the goods (can be owner or no owner).

Distributors / Importers / Traders (semi-processed goods) are companies which buy goods to resell them in their own name and on their own behalf.

Agents (semi-processed goods) are companies which do not have the power to conclude agreements in the name and for account of the principal but solely act as a representative of semi-processed goods.

Brokers are companies which bring buyer and seller together and are obtaining commission from one or both the parties.

Wholesalers / Distributors / Importers (consumer goods) are companies which buy packed consumer products and resell them in their name and on their behalf.

Agents (packed consumer products) are companies which do not have the power to conclude agreements in the name and for the account of the principal but solely act as a representative.

Retailers are companies which sell the packed consumer products to the final consumer.

Institutional Outlets are companies which supply consumer products to catering establishments.

National associations related to aquaculture sector.

Obligations

Accept all terms and conditions for SUPPORTING members according to the (NVCS / RQCS) statutes.

Adopt the parasites free environment reference defined (AENOR¿)

Acknowledge responsibility in assuring compliance with food law and labelling regulations.

Obliged when a problem arises to help solving the problem.

Rights

Approved SUPPORTING participants have the right:

To be represented as a Non-voting member in the "National General Assembly".

An optional right to obtain feed-backs on analyses and inspection-reports can be granted by a NVCS.

To receive early warning information provided by the EVCS

2.3 LEGAL ASPECTS

Statutes of the European VCS

Established by the EVCS and approved by the General Assembly.

Statutes of the National VCS

To be established by national associations.

2.4 FINANCIAL ASPECTS

The fees are decided and approved by the Executive Body and General Assembly.

Association need to be funded in a certain way. An agreement in the way to calculate fees need to be set.
As an example: volumes produced, consumption per country or economical figures can be useful as a valid indicator.

3. Guidelines

The following guidelines and points (sample taking, inspection, traceability, etc.) are based in the “Specific Regulations on Conformity Certification for production of AQUACULTURE

FISHERY PRODUCTS EXCLUSIVELY REARED IN AN ENVIRONMENT FREE OF VIABLE ANISAKIS" by AENOR (Spanish Association for Standardization and Certification).

3.1 SAMPLE TAKING

Objective

This guideline will provide the necessary minimum requirements for sample taking. This model can be followed in order to take into account for more zoonotic parasites which may be present in the aquaculture sector.

Scope

The sampling system will cover all products within the scope of the EVCS. Samples will be taken from:

Participants: from the plant and from the market.

Non-Participants: from the market (companies which do not participate in an EVCS).

Requirements

The system shall ensure that: As a minimum

Homogenous samples of sufficient volume to enable necessary analysis following AENOR guideline previously mentioned.

Each year a provisional sampling plan will be established with the following information:

1) Participants

Name of participant and minimun number of products should be taken according to the production volume or turnover for participant. The basis for the sampling plan per participant has to be documented.

For traceability reasons counter samples of final product have to be identified, sealed and stored. The linked documentation has to be studied. As in other counter samples systems, 3 samples should be taken. Each sample has to be identified with a unique sample code (number) and correctly labelled in order to record several information (for example: REGA nº, batch Nº, origin, sampling point and inspection).

2) Non-participants

In principle finished products from non EVCS-participants have to be checked.

Consequently, these controls should be planned on comparable basis to requirements for the participants (name of non-participant, number of samples to be taken and analysed).

The selection of these products and the total number of samples to be taken is left to the decision of each N/RQCS depending on the product scope of the non-participant and the local situation.

Samples have to be stored at appropriate conditions and handled according to requirements.

3.2 INSPECTION

Objective

This guideline provides minimum requirements for inspection.

Scope

Inspections are in principle possible for all participants.

Requirements

The system shall ensure that ordinary participants, independent of their sizes, are inspected at least once a year. Additional inspections are possible if necessary (e. g. in the first years of participation and in case of necessary traceability checks.).

The system has the right to carry out unannounced inspections. For practical reasons inspections can be announced (indicating the name of the inspector and week of inspection).

The Quality/Safety Assurance System of the participant is checked in accordance with the AEANOR guideline, these are some of the points mentioned in AENOR standard:

- traceability of products and accompanying documents;
- condition of the facilities and segregation of the cells/farms.
- counter samples
- fish feeding
- water
- the result of the corrective measures defined will be evaluated.

The inspector will establish a report in a standard form. The report has to be signed by the participants' representative and by the inspector.

3.3 IDENTITY AND TRACEABILITY

Objective

This guideline provides the minimum requirements for identification of all finished goods as well as accompanying documents in order to achieve full identity and traceability throughout the process of the participant and during the whole supply chain (from the sea to the consumer).

Scope

This guideline is valid for all products (see scope 1.3) purchased, manufactured and/or sold under control of EVCS.

Requirements

The participant shall ensure that all products are marked in such a way that full identity and traceability is guaranteed up to and including delivery to the customer, it is verified that relevant documentation is available to trace back finished products and all used raw materials to its sources in accordance with standards proposed (AENOR)

Corrective actions may be required. Corrective actions must be defined.

3.4 ANALYSIS BY INDEPENDENT LABORATORIES

Objective

This guideline provides the necessary minimum requirements for independent laboratories and analytical methods to use.

Scope

This guideline is valid for all laboratories engaged in analysis of products under EVCS or NVCS control.

Requirements

The laboratories shall ensure that:

- They are approved by a member of the EVCS or NVCS for defined analyses. Approval will be based on an evaluation of laboratory capacity, ability to work along accepted standards such as EN 45001 and successful participation in evaluation and validation ring-tests. The approval will be limited for a certain period of time.
- Incoming samples are properly registered, stored and otherwise handled. Samples are not allowed to be used for other purposes and institutions without prior written permission from the NVCS.
- Evaluation and interpretation of analytical figures and results are carried out in accordance literature and relevant food legislation.
- In particular cases other, relevant information can be used.
- All information received and results achieved are confidential and remain the property of the NVCS and are not to be used, distributed or communicated otherwise, without prior written consent from the NVCS.

The methods shall ensure that:

- Suggested minimum analysis for different types should be defined by EVCS system.
- Samples are analysed according to appropriate methods (see *ParaFishControl. Advanced Tools and Research Strategies for Parasite Control in European farmed fish. Deliverable D7.3 Elaboration of a Food Safety Programme)*.
- Results and evaluation should be reported in a standardised form within the time agreed upon between the NVCS and the laboratory.

3.5 EVALUATION OF ANALYTICAL FIGURES

Objective

This guideline provides the minimum requirements for evaluation of samples analysed in order to establish a uniform bases for approving products.

Scope

This guideline will cover all evaluations CAR (corrective action report).

3.6 REQUIREMENTS

The evaluation system shall ensure that:

- A first evaluation will be carried out by the analysing laboratory. The result of the evaluation and the analysis will be distributed to the responsible NVCS.

The provisional overall evaluation will be carried out by:

1. The NVCS Executive Body (provided that sufficient knowledge is available) on the basis of the first evaluation of the laboratory and own interpretation of all information obtained.

and/or

2. An expert committee who will evaluate the received analysis without knowledge of the first (laboratory) or provisional evaluation of the Executive Body.

In case of questionable results additional analyses will be carried out in order to clarify the reported deviations and if necessary additional information is requested from the participant concerned.

All evaluations of the analytical figures are based on the appropriate methods and other relevant information.

The final evaluation is made based on all results and information obtained and will be reported to the executive body of the national or regional system.

3.7 REPORTING

Objective

This guideline provides the necessary minimum requirements for reporting in order to enable an early warning and reporting system.

Scope

This guideline will cover all information exchanged between participants/national organisations and European organisations in respect to market observations, results of analyses, adulterations and execution of the national system.

Requirements

The system shall ensure that:

The European Executive Body will be informed by all members about observations of food safety issues.

The Executive Body of EVCS will collect, evaluate and distribute this information to all concerned via the national organisations.

The EVCS members will prepare a yearly report and sent to the EVCS Executive Body. This yearly report will include all data on

- Participants
- Finance
- Control activities
- Sample taking
- Analyses
- Non-conformities
- Corrective actions

The Executive Body of the European level will prepare a yearly status report about the European status as a synopsis of all Member reports for the General Assemblies.

On national level agreements have been made on the analytical feedback to participants.

3.8 CORRECTIVE ACTIONS

Objective

This guideline outlines the measures which form the basis for an effective corrective action system.
The only objective of corrective actions is to remove non-conforming products from the market and to avoid occurrence of the same problem in the future.

Scope

This system will cover all possible corrective actions on national and European level. Corrective actions can be imposed on participants or national organisations, where appropriate, in case of violation of:

- food law regulations
- the rules of the control system

Requirements

The system shall ensure that:
1. The Executive Body is free in the selection of the corrective actions to be taken depending on the severity and importance of the complaint.
2. The following corrective actions are available depending on local possibilities:

Informative letter to be used for probably accidental production faults with only low effect on the competition situation, with request for correction and improved quality assurance, announcement of post-controls.

Obligation acknowledgement for defined quality defects and distinct effect on the competition situation. Correction measures in the market and the company are to be agreed upon.

Payment of a penalty fee, agreement of a significantly higher penalty for each further case of repetition.

Suit before a public court.

Demand to call back the goods, possibly with support of third parties (limited to quality deviations that are harmful to one's health).

Impositions that are prerequisites for the further participation in the control system.

Warning letter with the information that expulsion from the system will be the consequence in case of repetition.

Withdrawal of the participant's certificate either for a limited or unlimited time.

Expulsion from the control system.

3.9 NATIONAL VCS APPROVAL

Objective

This guideline provides the minimum requirements for the approval or re-approval of national or regional control systems by the EVCS.

Scope

This guideline covers the basic conditions for the approval of all national or regional control systems.
Applying members are provisionally approved after a written confirmation to implement all the requirements in a period of maximum one year. Once a applicant has proven to fulfil all the requirements an approval will be sent in a written form.

Requirements

The EVCS shall ensure that the NVCS is fulfilling the following requirements:

- Providing a suitable formal organisation for running the system according to EVCS rules and guidelines to guarantee satisfying functioning of:
  - Participants plant inspection
  - Analytical control
  - Evaluation of results
  - Corrective measures
  - Reporting to EVCS
- Providing the financial basis for all member and participants obligations.
- Providing a documentation system in order to demonstrate proper functioning of the system which includes:
  - List of participants
  - Minutes from all organisation bodies
  - Sampling plan
  - Inspection reports
  - Analytical and evaluation reports
  - Taken corrective actions

3.10 APPROVAL OF PARTICIPANTS

Objective

This guideline provides the minimum requirements for the approval of participants.

Scope

This guideline is valid for all companies applying for participation in a national or regional control system. It specifies the basic conditions for approval.

Requirements

- The applying company will send in the application in writing by means of a standard form with the application it must be made clear that the applicant agrees with the objectives and rules and guidelines of the EVCS and national control system.
- A maximum period of one year is allowed for participants to demonstrate conformance.
  - During this time Provisional Approval will be granted, this approval expires without further notice.
  - If successful, full approval will be awarded for a period of one year and be subject to annual reassessment.
  - Continuation of the approval is dependent upon a continuation to fulfil the system's conditions.

The NVCS shall ensure that the participant, in addition to this general obligation to observe official legislation for due diligence, fulfils the following requirements of the EVCS:

- Designate an individual to be responsible for the implementation and/or supervision of the NVCS requirements
- Provision of documentation to clearly identify merchandise, supplier, use, customer, quantity, batch date, date i. e. traceability.
- Recording of all analyses carried out to ensure safety and/or identity.
- Keeping of sufficient reserved samples of goods purchased or sold.

The final responsibility rests with the participant of the system.

3.11 HANDLING COMPLAINTS ON IMPORTED CONSUMER GOODS

Objective

This guideline provides rules for handling complaints on imported consumer goods.

Scope

This guideline covers the NVCS (of import country) possibilities to handle such complaints internally or in co-operation with NVCS of exporting country.

Requirements

1. Each NVCS is responsible for the control and the assurance of all consumer goods offered/produced in its control area.
2. If imported consumer goods fail to meet the required standard, the NVCS which detected the case has two options:
   2.1. The NVCS has the right to handle the complaint in its own responsibility and take the needed corrective actions to stop disturbances in its domestic market (application of own catalogue of measures).
   2.2. The NVCS can hand over the problem to the NVCS where the producer concerned of the imported product is located. This NVCS can take further corrective actions according to its catalogue of measures.
3. In both possible cases the NVCS of the importing and exporting countries should inform each other and the EVCS-Secretariat (for information only) about all actions taken and the outcome of the case.

4. Legislation and References

- *COMMISSION REGULATION (EU) No 1276/2011 of 8 December 2011 amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards the treatment to kill viable parasites in fishery products for human consumption.*

- *AENOR. Specific Regulations on Conformity Certification for production of aquaculture fishery products exclusively reared in an environment free of viable anisakis.*

- *EFSA. 2010. Scientific Opinion on risk assessment f parasites in fishery products. EFSA J. 8 (4), 1543 (91 pp.)*

- *Norma UNE 173202: Marine Aquaculture. Marine fish farms. Design and operation.*

- *ParaFishControl. Advanced Tools and Research Strategies for Parasite Control in European farmed fish. Deliverable D7.3 Elaboration of a Food Safety Programme.*

5. Annexes

4.1 MEMBERS LIST

Members of EVCS

All members (company names) must be listed. The minimal details below as proposed

Member	Contact data
Company 1 **Adrdress**	Name Ph..: 00 11 22 33 44 55 Fax: 00 11 22 33 44 55 E-mail: mail@mail.com
Company 2 **Adrdress**	Name Ph..: 00 11 22 33 44 55 Fax: 00 11 22 33 44 55 E-mail: mail@mail.com

Members of the NVCS

Representative NVCS	Contact data
Representative NVCS 1	NAME Company: Company name E-mail: mail@mail.com
Representative NVCS 2	NAME Company: Company name E-mail: mail@mail.com
Representative NVCS 3	NAME Company: Company name E-mail: mail@mail.com
	NAME Company: Company name E-mail: mail
……	NAME Company: Company name E-mail: mail@mail.com

4.2 STATUTES

STATUTES

EVCS

European Voluntary Control System

Preamble

EVCS is the independent umbrella association of industrial self-control systems for national aquaculture control in the EEA and EU candidate countries. With its activities EVCS contributes to the safety and quality of the aquaculture products and to fair and safe competition in their markets. Thus the positive image, consumer confidence and economic performance of aquaculture products will be improved and strengthened.

Par. 1
Name, Registered Office, Financial Year, Legal Form and Term

1. The Association has the name "XXXXX European Voluntary Control System", in short form: EVCS.
2. Financial year is the calendar year.
3. The legal form for the association should be defined and register if needed.

Par. 2
Object of the Association

The object of the Association is to harmonise within the European Economic Area (EEA) and EU candidate country markets the food safety aspects of the aquaculture control systems and to support aquaculture manufacturers assuring the absence of zoonotic helminths in farmed seafood products.

1. In particular, it is the object of the Association to:
   - 1.1. To assure that the fishery products (derived from fish farming) do not represent a health hazard with regard to the presence of viable parasites.
   - 1.2. To assure the absence of zoonotic helminths in farmed seafood products.
   - 1.3. To protect the image of the products and the industry.
   - 1.4. To assure a fair competition in the sector.
   - 1.5. To develop control systems in countries where these not yet exist and to harmonise existing systems.
   - 1.6. To harmonise and to create consensus regarding the interpretation of analysis results on behalf of the members (the operational quality control systems).
2. For this purpose, the Association will:

2.1. promote the development of Systems in the EEA and EU candidate countries;
2.2. define the minimum requirements and guidelines for the proper functioning of Systems
2.3. work on the basis of respective EU legislation
2.4. approve the Systems after provision of evidence fulfilling the defined minimum requirements and then supervise their performance;
2.5. define the minimum requirements for the acceptance of participating aquaculture companies in the Systems.
2.6. coordinate the tasks of the Systems and maintain the flow of information. The Association cannot be held liable for information provided;
2.7. develop consistent principles for promotion of the Association, of the Systems and of the participating companies;

The Association may become a member of another association / organisation as far as it is permitted by law and approved by the General Assembly.

Par. 3
Membership

Membership is voluntary. Members are:

1. Ordinary Members:
   Associations within the EEA and EU candidate countries, considered as national control organisations which are running a control scheme (NVCS; Nathional Quality Control System).
2. Supporting members:
   Individual companies and other legal entities willing to support the work and philosophy of the Association and provide financial sponsorship.
   Application for membership will be made in writing. The Board of Directors will decide on membership at its next regular meeting. Should the Board of Directors reject an application, the applicant has the right to raise an objection at the next General Assembly. The decision of the General Assembly is final.

Par. 4
Obligations of Members

All members are obliged to:

1. Actively support the aims and objectives of industrial self-control and the Association;
2. Pay membership fees as specified in the contribution order;
3. In addition, ordinary members are obliged to:
   3.1. maintain and further develop a control scheme;
   3.2. comply with the Association's minimum requirements and guidelines for local control systems;
   3.3. actively participate in the bodies of the Association;
   3.4. regularly report on special observations in their markets and submit an annual report on control activities and results.

Par. 5

Rights of Members

1. The members are entitled to submit proposals to the Board of Directors and to the General Assembly.
2. All members will receive an annual activity report (in anonymous form) from the Association on the situation in the individual markets.
3. Ordinary Members have the right to be represented in all bodies of the Association.
4. Voting rights at the General Assemblies:
   4.1. Each NVCS has the right to vote. Each NVCS 1 vote.
   4.2. Supporting Members have the right to participate at the General Assembly without the right to vote.
   4.3. Members can represent only the vote(s) of one other member via a proxy.
   4.4. In no case one member can represent a majority of the votes.

Par. 6
Termination of the Membership

Membership can be terminated:

1. by written notification at least 12 months prior to the expiration of a financial year by registered mail;
   1.1 by exclusion as a result of a decision of the General Assembly in the case of a serious ground, e. g. in the case that:
   - a member commits a serious infringement of the Statutes
   - a member causes damage to the interests of the Association;
   - membership fee due remains unpaid for 6 months after its invoicing and a respective reminder.

   The Board of Directors can, if a member does not comply with point Par.6, 1.2, suspend the member until the final decision of the following General Assembly. Written objection against the suspension may be made within one month of notification. All membership rights are suspended in the interim.

Par. 7
Bodies of the Association

1. The bodies of the Association are:
   1.1 General Assembly;
   1.2 Board of Directors;
2. The members of the Board of Directors and the members of all other bodies will play an active role in promoting the aims and development of the Association. They will carry out their duties objectively and will refrain from all improper use of business or operating secrets related to the Association of which they become aware in the course of their activity.

Par. 8
General Assembly

1. The General Assembly is the highest ranking body of the Association. It consists of delegates of the members and will be called upon and presided over by the President.

2. Ordinary General Assembly

   The Ordinary General Assembly will be held once per year, normally within the first half of the year. The members are to be invited in writing at least 4 weeks prior to the meeting and the invitation is to include the agenda.

   The tasks of the Ordinary General Assembly are as follows:

   1.1 election and dismissal of the members of the Board of Directors, President and Vice-President;
   1.2 acceptance and approval of reports by the Board of Directors and the President;
   1.3 approval of the acts of the Board of Directors and the President/VicePresident;
   1.4 approval of EVCS guidelines and minimum requirements for the Systems;
   1.5 acceptance of a report on the status of the EVCS and the Systems;
   1.6 election and dismissal of a financial auditor;
   1.7 acceptance and approval of the financial reports;
   1.8 approval of the annual budget;
   1.9 approval of EVCS contribution order for members
   1.10 approval of using rules for the EVCS logo
   1.11 passing of resolutions on proposals submitted by members and/or the Board of Directors;
   1.12 passing of resolutions on amendments to the Statutes of the Association;
   1.13 deciding on appeals against the refusal of membership or the suspension/exclusion of a member;
   1.14 approval of all matters concerning an exclusive co-operation agreement regarding central raw material assurance and supplier control services
   1.15 approval of EVCS membership in another body;
   1.16 passing of a resolution regarding dissolution of the Association.

3. Proposals and requests to the ordinary General Assembly must be submitted by members to the Board of Directors no later than 3 weeks prior to the General Assembly. Proposals received will be forwarded together with the agenda.
4. Extraordinary General Assembly meetings may be called on request of the President, the Board of Directors or on request of at least one of the members. The invitation to the extraordinary General Assembly which has to include the agenda has to be sent to the members at least 10 calendar days prior to the assembly. Requests and proposals to extraordinary General Assemblies are to be addressed to the Board and all EVCS members not later than 7 calendar days prior to the meeting.
5. All General Assemblies are properly constituted, when at least 50 % of the votes and/or proxy votes are present. This represents a quorum.
6. In case of resolutions regarding amendments to the Statutes and/or the contribution order the General Assembly is only regarded as constituting the required quorum if reference is made to such in the invitation or, respectively, in the agenda.
7. Resolutions regarding the Statutes, the contribution order and the dissolution and liquidation of the Association must be adopted with a 2/3 majority of all member votes.
8. All other resolutions, proposals and requests to the General Assembly need for approval just a simple majority of votes present or represented.

Par. 9
President, Vice President and Board of Directors

President, vice president and board of directors duties need to be defined in the statutes, these listed below are some which could be used as an example:

1. The President and the Vice President jointly represent the Association judicially and non-judicially.
2. The term of office of all members shall be two years.
    a. The members of the Board of Directors are eligible for re-election.
3. The President and the Vice-President must have an ongoing engagement in a participating aquaculture company.
4. The Board of Directors shall consist of at least three persons.
5. All Board members should be willing and able to actively engage in the Board of Directors' duties. All functions exercised in the Board are unpaid. As a rule, membership in the Board is personal and bound to ongoing engagement in a company participating in a System.
6. The Board of Directors is responsible for maintaining the independence and credibility of the Association and upholding the principles of industrial self-control.
   The Board of Directors supervises and ensures proper and equal implementation of all minimum requirements for the Systems
7. Each member of the Board of Directors has one vote. The Board of Directors will pass resolutions by majority vote of those present. In the case of parity of votes, the decision will be made by the President.
   The meeting represents a quorum when the invitation was extended in proper and timely manner and the majority of the members elected are in attendance.
8. The Board of Directors can appoint an Advisory Board and/or ad-hoc commissions and working groups to process specific questions in accordance with Par. 10.
9. The Board of Directors will decide upon the approval of a control scheme. Approval for new member control schemes will be granted on a provisional basis. After a maximum of two years such provisional approval has to be reviewed on basis of an EVCS audit confirming a proper implementation of all EVCS minimum requirements for market and packer control systems. Respective re-approval audits and procedures will be conducted in three year intervals on basis of a respective guideline.

Par. 10
Advisory Board/Ad hoc Commissions and Working Groups

Advisory Board/Ad hoc Commissions and Working Groups duties and roles need to be defined in the statutes, these listed below are some which could be used as an example:

1. An Advisory Board, ad hoc commissions and working groups (the “Committees”) can be appointed by the Board of Directors.
2. Each member country has the right to be represented in all Committees. The delegate will be approved by the Board of Directors. Additional delegates and/or experts can participate if supported by a majority of members in the respective Committee.
3. Activity in the Committees is unpaid. As a rule, memberships are personal.
4. The Committees are not decision taking bodies of the Association but finalise their respective tasks with recommendations for approval by the Board of Directors and/or the General Assembly.

5. It is the task of these Committees to advise the Board of Directors, in particular, in matters relating to:
   5.1 improvement of the control work, methods and procedures of the Systems;
   5.2 evaluation of results and reports on local market and fish farms controls,
   5.3 compilation of an annual control report of the member control systems;
   5.4 evaluation of the work of collaborating laboratories;
   5.5 technical matters of common interest or arising between members;
   5.6 development and maintenance of an early warning system for the sector;
   5.7 other tasks if and as defined by the Board of Directors;

Par. 11
Records/Language of the Association

All resolutions passed at meetings and/or conference calls are to be recorded in the minutes of the meeting, to be signed by the person chairing the meeting in question and passed on to the members of the appropriate body no later than four weeks after the meeting. The minutes will be approved at the next meeting of the body in question.

The official language of the Association is English and shall be the working language of the Association. These Statutes shall be written in English languages.

Par. 12
Dissolution and Liquidation

2. Dissolution is effected by resolution of the General Assembly in accordance the Statutes.
3. Liquidation is effected by the President or, as appropriate, by another liquidator appointed by the General Assembly.
4. Following completion of pending business and settlement of all liabilities the assets of the Association will have to be allocated to a non-profit purpose.