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Qualitative risk assessment of introduction of anisakid larvae in Atlantic salmon (*Salmo salar*) farms and commercialization of products infected with viable nematodes

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1 Keywords: Qualitative risk assessment, Anisakis, Atlantic Farmed Salmon, Fishery products, Food safety, parasitic
2 zoonoses, fish-borne disease, Pseudoterranova, fish-borne, food safety

3 **ABSTRACT**

4 In this study, the likelihood of introduction of anisakid larvae in Atlantic salmon (*Salmo salar*) farms and subsequent
5 commercialization of fish infected by at least one vital parasite was formally assessed using an adaptation of the risk
6 assessment framework for importation of animals and animal products of the World Organization for Animal Health (OIE).
7 The different plausible pathways were identified and outlined. The most recent information concerning the parasite, its
8 definitive, intermediate hosts and the farming practices typical of Atlantic salmon farms were reviewed and used to assess
9 the likelihoods of each key step along the pathways. A matrix for the conditional probabilities was adopted to combine
10 the qualitative estimations and obtain an objective and transparent overall risk of introduction along each route. In order
11 to avoid misinterpretation and overconfidence on the outcome, the uncertainties surrounding the estimations were
12 considered. The only situation for which the assessed risk in a typical Atlantic salmon farm was deemed to be non-
13 negligible involved the ingestion of infected hosts that penetrate the harvesting cages. In this event, the overall risk was
14 estimated as 'Very Low' with a high degree of uncertainty because of the scarcity of information in some of the key steps
15 along the pathway. However, the scientific evidence in support of the overall estimation suggests that the availability of
16 additional data would be unlikely to increase the final estimated risk. On the basis of the available information of the
17 system, the estimated risk of introduction and commercialization of farmed Atlantic salmon infected by viable
18 nematodes resulted 'Very Low' even under a conservative approach. The proposed qualitative model is an objective and
19 transparent method to assess the risk when data and information are scarce and provides a framework for the qualitative
20 assessment of the introduction of alive parasites in aquaculture/mariculture implants and subsequent commercialization
21 of infested fishery products. The framework could be easily adapted to other parasite-host interactions besides anisakid
22 nematodes in Farmed Atlantic salmon.

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29 **1. INTRODUCTION**

30 Fish-borne parasitic zoonoses represent a global emergent threat, among these, anisakidosis has shown widespread
31 expansion in the last two decades (Chai, Darwin Murrell, & Lymbery, 2005). The family Anisakidae includes zoonotic
32 parasitic nematodes among which, the species belonging to the genera *Anisakis* and *Pseudoterranova* are the most
33 commonly associated with infection in humans due to consumption of raw or undercooked fishery products. As a result
34 of their impact on the commercial value of fishery products and on public health, anisakids have become an important
35 economic and a public health concern worldwide (M. T. Audicana & Kennedy, 2008; Bouree, Paugam, & Petithory, 1995;
36 EFSA, 2010; Skirnisson, 2006).

37 The life cycle of Anisakidae of zoonotic interest is completed in seawaters and involves marine mammals (cetaceans and
38 pinnipeds) and piscivorous birds as definitive hosts. In natural conditions, the predation of infected fish leads to
39 bioaccumulation along the predation chain resulting in the risk of infection being higher in top predator fish species
40 (Strømnes & Andersen, 2003a) which as a result pose a higher risk for human health.

41 In order to prevent and control transmission of fishery product-borne parasites, the Section VIII of Annex III to Regulation
42 (EC) No 853/2004 lays down provisions for fishery products to be consumed raw or almost raw. The Regulation indicates
43 that fishery products intended to be eaten after a process that is not sufficient to inactivate nematode larvae must be
44 frozen at a temperature of not more than -20°C in all parts of the product for not less than 24 hours.

45 In April 2010, the European Food Safety Authority (EFSA) published a scientific Opinion on risk assessment of parasites in
46 fishery products (EFSA, 2010) providing criteria for determining the conditions under which fishery products from
47 aquaculture can be recognized as being free of viable parasites that may represent a hazard for human health. With
48 particular reference to farmed Atlantic salmon (*Salmo salar*), the Opinion concluded that farmed Atlantic salmon reared
49 in floating cages or onshore tanks and fed with compound feedstuffs are unlikely to contain live parasite. However, the
50 Panel on Biological Hazards did not considered routes of infection other than the feed and the risk was never assessed
51 formally.

52 Following that Opinion, in 2011, the Regulation (EC) No 1276/2011 modified the requirements set out in Annex III, Section
53 VIII, Chapter III, Part D of Regulation (EC) No 853/2004 allowing food business operators to not apply freezing treatment
54 if procedures approved by the competent authority are used to verify that the product does not represent a health hazard
55 with respect to viable parasites.

56 In the present study, the general approach recommended by the World Organization for Animal Health (OIE) for the
57 assessment of the risk posed by the importation of live animal and animal products (OIE, 2010) was adapted to formally
58 investigate the potential for live zoonotic nematodes to represent a risk for human health in farmed Atlantic salmon.

59 2. MATERIALS AND METHODS

60 For purpose of this study, the approach proposed by the OIE for the assessment of risks associated with the importation
61 of animals or animal products (OIE, 2010) was adopted. According to the OIE guidelines, the identification of the hazard
62 precedes the risk assessment, which is composed of three components: (i) Release assessment, (ii) Exposure assessment
63 and (iii) Consequence assessment. Generally, the final risk estimate is the result of the integration of the three steps but
64 because of the purpose of this study, the consequences of the commercialization of the infected fish are not considered.
65 The risk question being assessed was therefore formulated as “what is the likelihood of an Atlantic salmon (*Salmo salar*)
66 harvested from a typical farm being infected by at least one nematode of genus *Anisakis*, and subsequently
67 commercialized for human consumption”.

68 The different pathways describing the sequence of sufficient and necessary events leading to the introduction of the
69 parasite into a fish farm were outlined and the likelihoods of introduction assessed for each pathway considering the
70 farming practices typical of Atlantic salmon.

71 Qualitative risk assessment models foresee the use of subjective risk levels to describe the likelihood of unwanted events;
72 in this work, the qualitative terms proposed by Kahn et al. (S.A. Kahn et al., 1999a; S.A. Kahn, Wilson, Perera, Hayder, &
73 S.E., 1999b) were adopted (Table 1).

74 *Table 1 Definition of the qualitative terms used to describe the likelihoods of the necessary events leading to the introduction of anisakid*
75 *nematodes into an hypothetical Atlantic salmon farm and commercialization of infested products (after Kahn et al.1999).*

Likelihood	Decription
High (H)	Expected to occur
Moderate (M)	Occurrence less than 50% probability
Low (L)	Unlikely to occur
Very low (VL)	Rarely occur
Extremely low (EL)	Very rarely occur
Negligible (N)	Chance of occurrence so small that can be ignored

76
77 The biological and epidemiological characteristics of the parasite, its primary and accidental hosts together with the
78 biosecurity practices applied in Atlantic salmon’s farms were reviewed and discussed to assign the likelihood at each step
79 along each identified pathway. Information and evidence included in this assessment were collected from peer-reviewed

80 articles and from industry data reported in the environmental public reports published by the main producers of farmed
 81 Atlantic salmon worldwide. In each pathway, the likelihoods assigned to each event were combined to derive the overall
 82 estimate of the risk of introduction of the parasite in farmed salmon and commercialization of infested products. The risk
 83 estimates were expressed as cumulative likelihoods obtained combining the qualitative estimates of the inputs according
 84 to the matrix of conditional probabilities presented in table 2 and previously applied by EFSA and in other qualitative risk
 85 assessments (EFSA, 2007; Peeler & Thrush, 2009).

86 *Table 2 Combination matrix used for the estimation of the conditional likelihoods. The product of two probabilities is always less than*
 87 *the lowest probability and is sometimes given as a range (e.g. N-EL). However, as explained in the EFSA report, since qualitative terms*
 88 *cover a wide range of likelihoods, the combined estimate is, in some cases, equal to the lower estimate (e.g. a step 'n' with an estimate*
 89 *of VL with a step 'n+1' with an estimate of EL produces and an overall estimate of N-EL).*

Likelihood step 'n+1'	Conditional likelihood step 'n'					
	N	EL	VL	L	M	H
H	N	EL	VL	L	M	M
M	N	EL	VL	VL	L	M
L	N	EL	EL	VL	VL	L
VL	N	N-VL	EL	EL	VL	VL
EL	N	N	N-EL	EL	EL	EL
N	N	N	N	N	N	N

90
 91 This matrix defines a likelihood estimate for any binary combination of conditional events. If two or more independent
 92 risk factors contributed to the likelihood estimation for a single step, the likelihoods for each factors were estimated and
 93 the same matrix for the conditional probabilities was used to outline the overall likelihood for the step.

94 In order to avoid overconfidence on the outcomes and prevent misinterpretation, an assessment of the uncertainty
 95 surrounding each estimation was also carried out (Table 3), and expressed as: High, (H) Moderate (M) or Low (L).

96 *Table 3 Definition of the qualitative terms used to define the levels of uncertainty surrounding the likelihoods assigned to the steps along*
 97 *the pathways.*

Uncertainty	Interpretation
Low (L)	The estimation is strongly supported by data-evidence. Agreement by different authors
Medium (M)	The estimation is supported by few or incomplete data. Some authors report slightly different conclusions to others
High (H)	The estimation is supported only by scarce data or it is based on hypotheses not yet proved. Strong disagreement from different authors

98 With respect to the uncertainties, the worst estimate was conservatively considered among the risk factors and along the
 99 steps of the pathways; in this way, a high uncertainty in one level is enough to lead to a high uncertainty in the overall

100 outcome. An exception was made if the occurrence of the event in step n+1 is *Negligible* with *Low* uncertainty.

101 2.1 Hazard identification and characterization.

102 The different species belonging to genera *Anisakis* and *Pseudoterranova* are not reliably distinguishable morphologically
103 but several species have been identified at molecular level (S. Mattiucci et al., 1997; Simonetta Mattiucci et al., 2005;
104 Paggi et al., 2000; Paggi et al., 1991). The morphospecies most commonly associated to human infection are:

105 (i) *Anisakis simplex*, worm-like parasite, with larvae usually measuring 1 - 3cm length, thin, characterized by a
106 pinkish-white colour. It usually appears rolled up on itself. Larvae are normally localized in the viscera where
107 they are generally easily visible but can migrate into the muscle or the abdominal wall where the parasite is
108 more difficult to identify, especially in white fish (EFSA, 2007);

109 (ii) *Pseudoterranova decipiens*, worm-like parasite, larvae usually measuring 1 - 4cm length and characterized
110 by a reddish-brown colour it tends to present a large-rolled coil. In infested specimens it is usually located
111 at muscular level (McClelland, 2002).

112 The life cycle of the species belonging to genus *Anisakis* takes place in seawater and proceeds in several steps. In the first
113 step, eggs, are released in seawater with the faeces of the definitive hosts (mainly cetaceans such as whales, dolphins
114 and porpoises); in marine environment they develop to second stage larva and hatched in seawater (Anderson, 2000;
115 Kjøie, Berland, & Burt, 1995; McClelland, 2002; Smith & Wootten, 1978).

116 Newly hatched larvae can survive in marine environment for weeks and be eaten by a wide range of different
117 intermediate hosts (crustaceans and molluscs) where they develop to third stage larvae (L3). When fish or cephalopods
118 eat intermediate infected hosts, the L3 migrates to the coelomic cavity of the predator, which acts as paratenic host
119 since the parasite does not further develop. Paratenic hosts can acquire larvae by ingesting intermediate and paratenic
120 hosts leading to bioaccumulation along the predation chain; consequently, big and/or old fish and at the top of food
121 chain may host even thousands of nematodes (Smith & Wootten, 1978). Humans act as accidental hosts when they eat
122 undercooked infected fish or squid.

123 The larval stages and the biological cycle of *Pseudoterranova spp.* do not differ from the ones described for *Anisakis spp.*
124 even though the definitive hosts are usually pinnipeds like sea lions or seals instead of cetaceans. Moreover, larvae of
125 *Pseudoterranova.spp.*, do not have cuticular sheaths with lateral extremities that increase the buoyancy, thus, conversely
126 to larvae of *Anisakis spp.*, are not able to swim (Palm, 1999).

127 Although the dynamics underlying the geographical distributions of the most important intermediate and paratenic hosts

128 of *Anisakis spp.* and *Pseudoterranova spp.* are complex and still largely unknown (EFSA, 2010), considering the differences
129 in the habitat of the hosts involved; it is generally recognized that *Anisakis spp.* have an essentially pelagic life-cycle,
130 whereas *Pseudoterranova spp.* have a more benthic habit. Consequently, with particular reference to the Atlantic salmon,
131 parasites belonging to the genus *Anisakis spp.* represent a greater concern than *Pseudoterranova spp.* (Wootten, Yoon,
132 & Bron, 2010).

133 Following these considerations, nematodes of genus *Anisakis* were formally identified as the hazard of interest while
134 *Pseudoterranova spp.* is not considered further.

135 **2.1.1. Hazard characterization *Anisakis spp.* – Prevalences.**

136 The prevalences of the parasite in different wild fishes and data related to the occurrence of the different species of
137 genus *Anisakis* in infected fish show high variability according to both geographical region and hosts species (EFSA, 2010;
138 Mladineo & Poljak, 2014; Piras et al., 2014; Karl Marx A. Quiazon, Tomoyoshi Yoshinaga, & Kazuo Ogawa, 2011). The
139 complexity of the dynamics of infection leading to different proportions of the parasite's species in different hosts and in
140 different areas led to the cautionary conclusion that none of the fishing areas worldwide should be considered as *Anisakis*-
141 free, and thus, all the wild saltwater fishery products must be considered potentially infested (EFSA, 2010). For this
142 reason, geographical heterogeneities were not considered in this assessment.

143 **2.1.2. Hazard characterization *Anisakis spp.* – Pathogenesis.**

144 In humans, the accidental ingestion of live nematodes belonging to the family of Anisakidae, causes parasitic zoonosis
145 known as anisakidosis or anisakiasis, described for the first time in 1960 by Van Thiel (Van Thiel, Kuipers, & Roskam, 1960).
146 The minimum infectious dose is a single nematode (A. Daschner, Alonso-Gomez, Cabanas, Suarez-de-Parga, & Lopez-
147 Serrano, 2000; FDA, 2012). After ingestion, viable larvae may be excreted with the faeces after up to 48 hours with the
148 faeces, or result in the acute form of anisakidosis, the most frequently observed form, characterized by violent abdominal
149 pain, nausea and vomiting due to the larval inflammation of the intestinal mucosa. (Asaishi, Nishino, Totsuka, Hayasaka,
150 & Suzuki, 1980; Sakanari & McKerrow, 1989; Sugimachi, Inokuchi, Ooiwa, Fujino, & Ishii, 1985). The acute form might
151 degenerate into chronic if misdiagnosed or untreated. In the chronic form, the larvae penetrate the gastrointestinal
152 mucosa, causing the formation of granulomas with eosinophilic infiltrate and even abscesses. Granulomas and the
153 inflammation process remain even after the death of the worm that in the human body usually happens 3 weeks after
154 ingestion. Complications have rarely been reported in the literature and include a few episodes involving intestinal
155 obstruction (Sasaki, Fukumori, Matsumoto, Ohmori, & Yamamoto, 2003), colic intussusception (Furukawa et al., 2014;

156 Yorimitsu et al., 2013) and pneumoperitoneum (Ito et al., 2007). Moreover, the consumption of fish harbouring dead
157 *Anisakis spp.* larvae has been reported to be potentially dangerous because of possible allergic reactions (M. a. T.
158 Audicana, Ansotegui, de Corres, & Kennedy, 2002).

159 **2.2 Release and Exposure assessment for the Introduction of *Anisakis spp* into an Atlantic salmon farm**

160 The risk of the introduction of *Anisakis spp.* into an Atlantic salmon farm was assessed considering five pathways:

- 161 1. *Introduction through juveniles of wild Atlantic salmon captured for farm production*
- 162 2. *Introduction through feed contaminated with viable *Anisakis spp.* larvae*
- 163 3. *Introduction through wild salmon that have accidentally entered the floating cages*
- 164 4. *Introduction through escaped salmon that have been infected offshore re-entering the cages.*
- 165 5. *Introduction through the ingestion of infected hosts*

166 For each pathway the sequence of sufficient and necessary events leading to the release of the parasite into a generic
167 farm were identified and are outlined in figures 2-5.

168 **2.2.1 Pathway 1: Introduction through juveniles of wild Atlantic salmon captured for farm production**

169 The occurrence of anisakid larvae in wild salmon is known to be high and above 70% (EFSA, 2010; FDA, 2015), therefore,
170 the harvest of wild animals could represent an important pathway for the commercialization of risky products. However,
171 unlike the farming methods applied for other species (i.e. Cod or Eels), the production cycle of Atlantic salmon is totally
172 closed and the capture of juveniles does not occur. This pathway was not further explored.

173 **2.2.2 Pathway 2: Introduction through feed contaminated with viable *Anisakis spp.* larvae**

174 The likelihood of the introduction of the parasite into a hypothetical farm through the feed depends on: (i) the source and
175 the nature of the raw material and (ii) the thermal/physical treatments to which raw material has been subjected.

176 The scenario trees outlined in Figure 1 represent the pathways leading to the introduction of the parasite into a generic
177 farm by feed. Both the use of live feed (A) and treated feed (B) were considered.

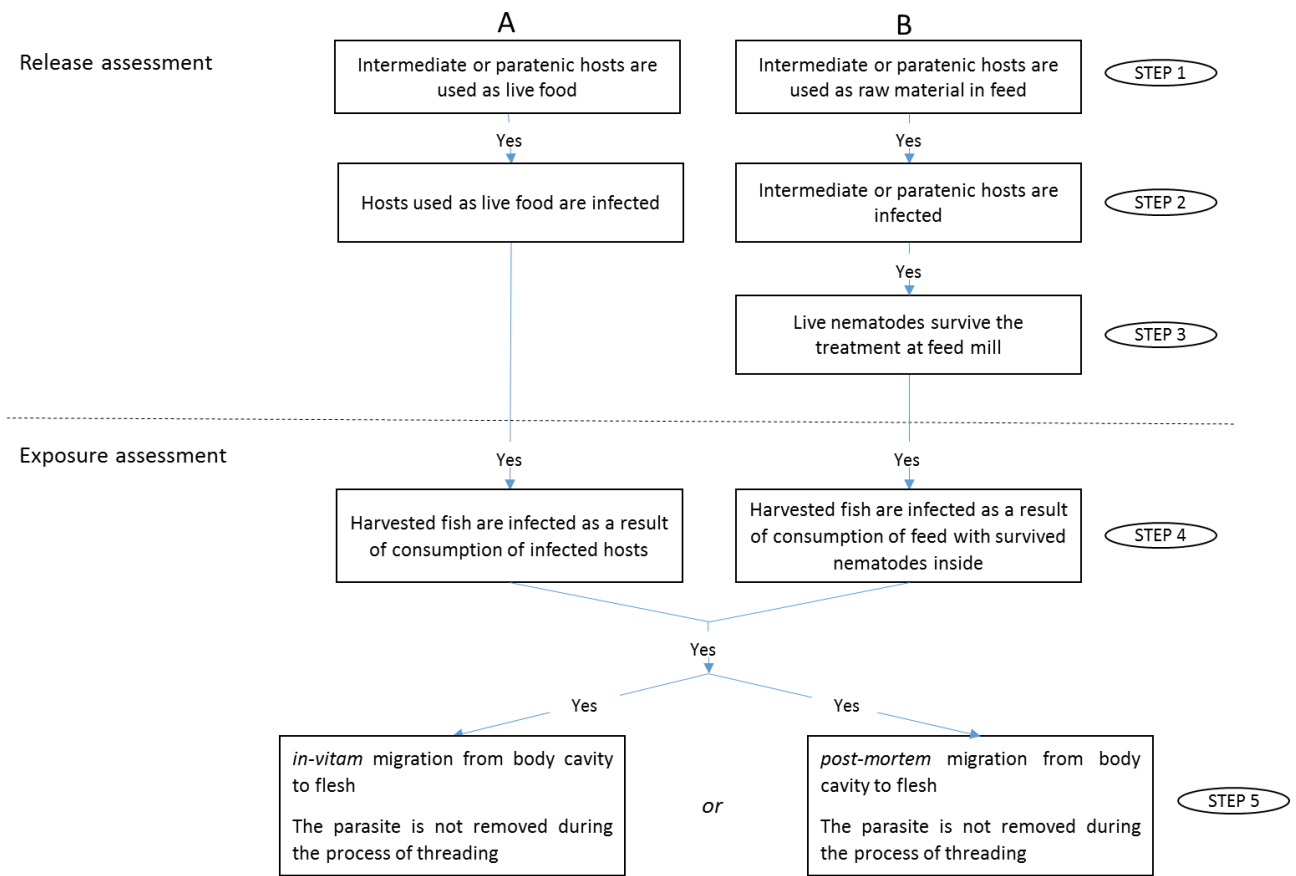
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179

180

181 Figure 1. Pathway nr.2; flowcharts outlining the required steps for the introduction of *Anisakis spp.* into a hypothetical farm through
 182 feed. Both live food (pathway A) and feed were considered (pathway B).

183



184

185

186 The use of live feed would lead to an evident risk of introduction of the parasite (Wootten & Smith, 1975), however, the
 187 farming of the Atlantic salmon foresees the use of heat-treated feed only; therefore, the pathway A was not considered.
 188 Moreover, since farmed salmon are fed with composite feed which includes wild species like herring (*Clupea harengus*),
 189 capelin (*Mallotus villosus*), Chilean anchovies (*Engraulis ringens*) etc., the first step of pathways B was considered as an
 190 event that always occurs, and its likelihood was not included in the assessment.

191 **Step 2.** Considering the wide range of intermediate/paratenic hosts species to which *Anisakis spp.* have adapted (EFSA,
 192 2010; Klimpel, Palm, Rückert, & Piatkowski, 2004), together with the variability and the uncertainty underlying the
 193 presence of the parasite in wild species (Section 2.2.1); the likelihood of *Anisakis spp.* being present in wild species used
 194 as raw material for the farmed salmon feed is considered **High** with **Low** uncertainty.

195 **Step 3.** Farmed salmon are fed with dry pellet produced by extrusion and temperature above 150°C. The likelihood of
 196 parasite surviving the treatment is **Negligible** with **Low** uncertainty.

197 **Step 4.** Following the processing treatments at feed mill, the next hypothetical step is the consumption of feed containing
198 viable larvae. The conservative likelihood assigned to this step is **High** with **Low** uncertainty.

199 **Step 5.** At this stage, the combined likelihood of the parasite migrating (*intra-vitam* and/or *post-mortem*) from the
200 coelomic cavity to edible muscles and not being removed during the process of threading is assessed.

201 The *intra-vitam* migration of the parasite is not a certain event and the frequency distributions of anisakid third stage
202 larvae in hosts' tissues are believed to be affected by a number of conditions encountered within the hosts themselves
203 (Strømnes & Andersen, 1998) among which, the lipid content is believed to play an important role (Strømnes, 2014;
204 Strømnes & Andersen, 2003b).

205 Several recent studies reported the presence of anisakid nematode larvae in muscles surrounding the body cavity of
206 freshly caught salmonids (Karl, Baumann, Ostermeyer, Kuhn, & Klimpel, 2011; Senos, Poppe, Hansen, & Mo, 2013;
207 Setyobudi, Jeon, Lee, Seong, & Kim, 2011; Urawa & Fujisaki, 2006) or sibling species (Karl Marx A Quiazon, Tomoyoshi
208 Yoshinaga, & Kazuo Ogawa, 2011) indicating that the *intra-vitam* migration of the parasite is an event that it is likely to
209 occur in salmonids. Following these considerations, the estimated likelihood of parasite *intra-vitam* migration from the
210 coelomic cavity is considered to be **High**, with **Low** uncertainty.

211 The *post-mortem* migration of the parasite from the viscera to flesh is still a debated topic, the scientific opinion from the
212 Panel on Biological Hazards (EFSA, 2010) reported: "... based on scientific evidence it is not clear when, under what
213 conditions and in which fish species, *post-mortem* migration of *Anisakis simplex* larvae occurs ...". However, factors
214 stimulating the migration of the parasite after the death of the host are presumably related to physico-chemical changes
215 in viscera (Smith & Wootton, 1975) and time-temperature storage conditions (Chen, Cheng, Grabner, Chang, & Shih,
216 2014; Cipriani et al., 2015). At this respect, it should be considered that opposite to wild salmon, reared fishes are
217 processed immediately after collection from the floating cages; consequently, the likelihood of *post-mortem* migration in
218 farmed Atlantic salmon is considered as **Negligible** with **Low** uncertainty. The worst scenario (*intra-vitam* migration) was
219 conservatively considered in this step.

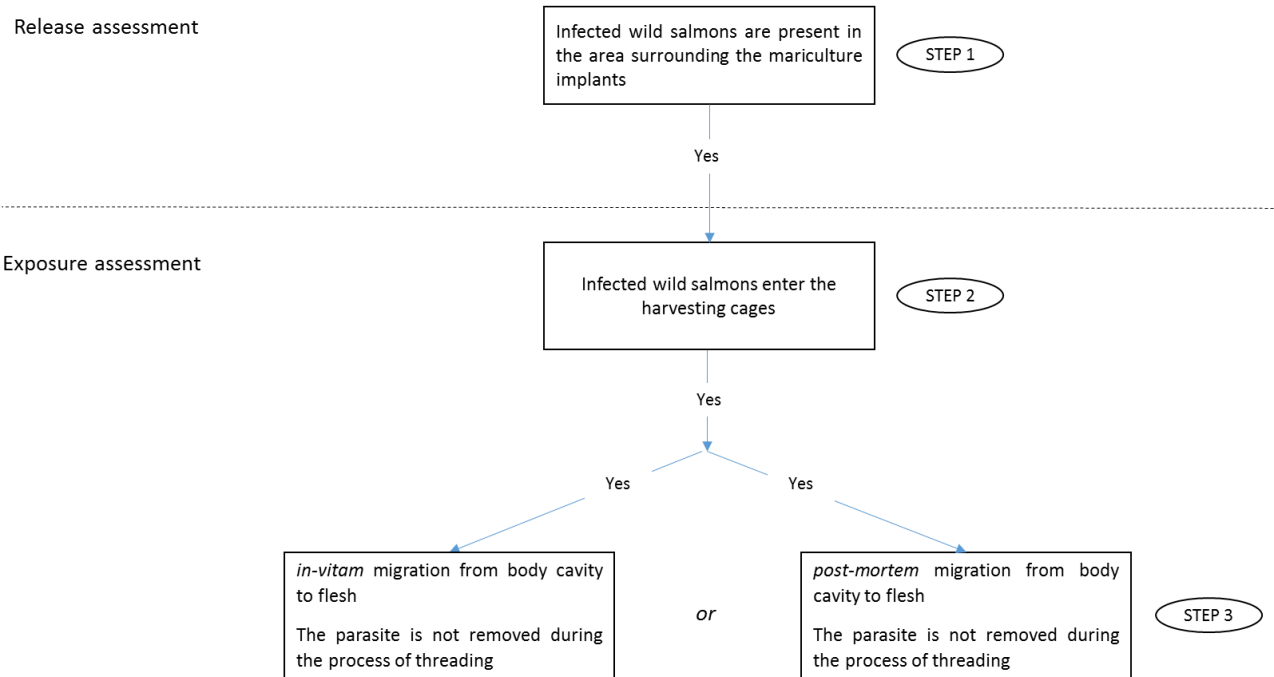
220 2.2.3 Pathway 3: Introduction through wild salmon that have accidentally entered the floating cages

221 The pathway leading to the introduction of *Anisakis spp.* by the accidental entry of wild salmon into the floating cages is
222 outlined in Figure 2.

223

224 Figure 2 Pathway nr.3; flowchart outlining the required steps for the introduction of *Anisakis* spp. into a hypothetical Atlantic salmon
 225 farm and commercialization of infested products by accidental entry of wild salmon in floating cages.

226



227

228 **Step 1.** As mentioned in section 2.2.1. The occurrence of *Anisakis* in wild salmon is known to be high; however, although
 229 the presence of wild salmon in the areas surrounding the salmon farms cannot be excluded, a low density of wild salmon
 230 is usually recorded in the areas bordering the mariculture implants (Ford & Myers, 2008; Frazer, 2009). From these
 231 evidence, the estimated likelihood of wild salmon being present in the area surrounding the farm is **Low** whilst the
 232 likelihood of wild salmon being infected if present is **High**; consequently, the combined likelihood for the presence of
 233 infected wild salmon in the area surrounding the farms is **Low**. Because of the scarcity of available data to assess the
 234 salmon distribution variability across the geographical areas, the level of uncertainty was considered **Medium** for the first
 235 condition and **Low** for the second one leading to an overall conservative **Medium** level of uncertainty for this step.

236 **Step 2.** Atlantic salmon are grown to marketable size in floating nets offshore; the possibility for a wild salmon to
 237 penetrate the harvesting nets and to mingle with the reared salmon is linked to the presence of a hole in the floating
 238 cages. However, the first consequences of a hole in a floating net would be the escape of the raised fish, with obvious
 239 economical loss and huge environmental consequences (Crozier, 1993; Fraser, Cook, Eddington, Bentzen, & Hutchings,
 240 2008; Gausen & Moen, 1991; Thorstad et al., 2008); consequently, it is of industry interest to apply all the biosecurity
 241 measures aimed to prevent/avoid the escape of the reared fishes, and indirectly, the introduction of wild animals. At this
 242 respect, the major food business operators in salmon harvesting invest many resources to pursue the so-called 'zero

243 escape' objective and public reports shown how the efforts resulted in a steadily decreasing occurrence of incidents
244 leading to 'escape' events (Lerøy Seafood Group, 2012, 2013, 2014; Marine harvest, 2012, 2013, 2014).

245 Moreover, incidents resulting in an escapes should not be interpreted as events favouring at the same time the
246 introduction of wild individuals. In fact, all the reported incidents were one-way oriented in determining the 'escape'
247 without favouring the 'introduction' in any way.

248 In order for an introduction to take place, any breakage in the netting would need to be of a size insufficient to allow a
249 large-scale release of farmed fish, which would be noticed immediately by the operators on underwater surveillance
250 cameras. Conversely, this underwater surveillance system reduces the likelihood of ingress of wild salmon to the cages,
251 since any damage in netting is likely to be relatively small in size.

252 Following these considerations, the estimated likelihood of the wild salmon accidentally penetrating the floating cages is
253 *Negligible* with *Low* uncertainty.

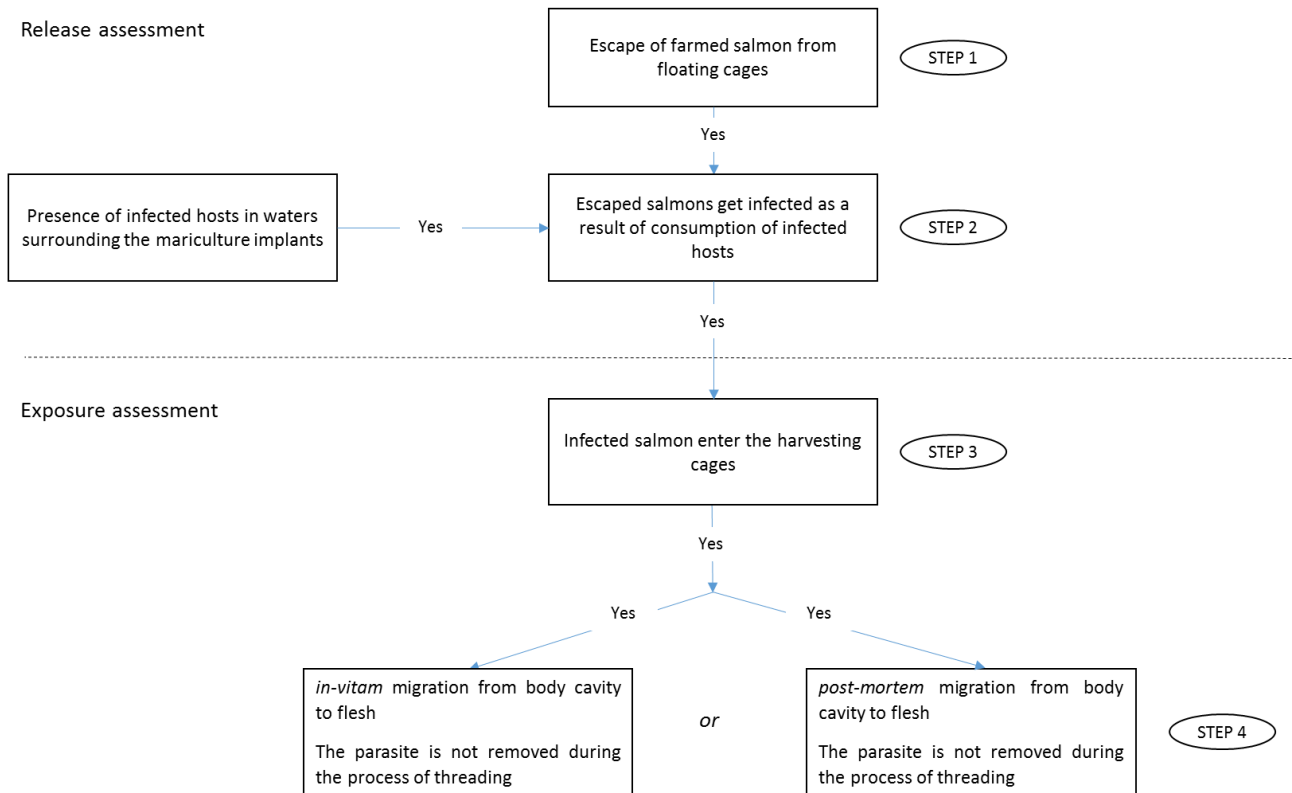
254 **Step 3.** Following the considerations in Section 2.2.2. (Step 5), the estimated likelihood of anisakid larvae migrating in
255 flesh from the coelomic cavity is *High* with *Low* uncertainty.

256 **2.2.4 Pathway 4: Introduction through escaped salmon that have been infected offshore re-entering the cages.**

257 Although the 'escape' events are rare (Section 2.2.3, Step 2), the likelihood of the re-introduction of escaped salmon that
258 have been infected offshore is assessed (Figure 3).

259 Figure 3 Pathway nr.4; flowchart outlining the required steps for the introduction of *Anisakis spp.* into a hypothetical Atlantic
 260 salmon farm and commercialization of infested products by escaped salmon that have been infected offshore re-entering the cages.

261



262

263 **Step 1.** From consideration discussed in Section 2.2.3. (Step 2), the estimated likelihood of farmed Atlantic salmon
 264 escaping in seawaters is **Low** with **Low** uncertainty.

265 **Step 2.** *Anisakis spp.* larvae can survive in seawaters for extended periods and be eaten by a wide variety of different
 266 hosts. Although the parasite mainly uses *euphausiids* (krill) living in deeper water offshore as first intermediate host
 267 (Smith, 1983), the parasite is able to select host species depending on the locality (Klimpel et al., 2004). Therefore, the
 268 estimated likelihood for the presence of infected hosts in the areas bordering the implants is **High**, with a **Low** uncertainty.
 269 Since escaped salmon are forced to prey to survive, the estimated likelihood of escaped salmon preying infected hosts
 270 is **High** with **Low** uncertainty.

271 **Step 3-4.** Estimated likelihoods and uncertainties for these steps are identical to the ones reported in pathway 3.

272 **2.2.5 Pathway 5: Introduction through the ingestion of infected hosts**

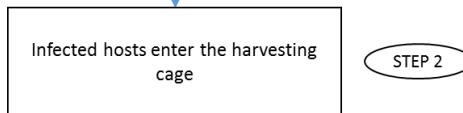
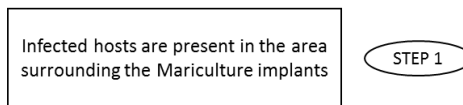
273 The pathway leading to the introduction of *Anisakis spp.* by ingestion of infected hosts is outlined in Figure 4.

274

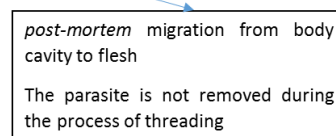
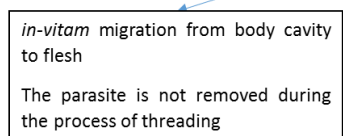
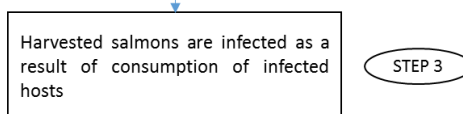
275 Figure 4. Pathway nr.4; flowchart outlining the required steps for the introduction of *Anisakis* spp. into a generic farm by ingestion
276 of infected hosts.

277

Release assessment



Exposure assessment



STEP 4

278

279

280 **Step 1.** Following considerations in Section 2.2.4. (Step 2), the estimated likelihood of infected hosts being present in
281 areas bordering the implants is **High** with **Low** uncertainty.

282 **Step 2.** The access of infected hosts to the floating cages is strictly dependent on the size of the hosts themselves. In fact,
283 while the introduction of large hosts is physically prevented by the meshes' size, no barriers are applicable to hosts smaller
284 than the meshes. Thus, the estimated likelihood for this step is **Medium**, with a **High** level of uncertainty due to the lack
285 of information about the occurrence of the different host species (with particular interest in host size) in the area
286 bordering the implants.

287 **Step 3.** In this step, the ingestion of anisakid larvae is linked to the predation of the hosts. Even though farmed salmon
288 are fed with dry pellet, the results of two recent studies (Marty, 2008; Mo et al., 2014) reported the presence of *Anisakis*
289 *spp.* larvae in runts of farmed Atlantic salmon (Fishes with clear signs of poor performance and/or abnormal appearance,
290 emaciated and not suitable to be marketed for human consumption), suggesting that farmed salmonids in open cages
291 may in some cases feed on live food. However, it is important to emphasize that the nematodes were found so far only

292 in underweighted or discarded animals and never in salmons processed for human consumption. The authors explained
293 their findings hypothesizing that in floating cages weak animals undergo competition phenomena that limit their access
294 to feed and thus, runts must feed with 'anything' that can be eaten in order to survive. Based on this evidence, it is
295 assumed that the likelihood of salmons processed for human consumption (i.e. not classified as 'runts') preying live food
296 to supplement their feed intake is **Low**. A **High** uncertainty is cautiously assigned because of the scarcity of data supporting
297 the assessment.

298 **Step 4.** As discussed in Section 2.2.2 (Step 5), the presence of the parasite in infected salmons represents a risk for human
299 health only if the parasite is not physically removed during the process of threading. According to previous estimations,
300 the likelihood of the event is **High** with **Low** uncertainty.

301

302 **3. RESULTS**

303 The estimated likelihoods and uncertainties for each step combined through the Combination matrix (Table 2) provided
 304 the cumulative likelihoods; these are reported for each of the pathways considered in tables 4-7 with estimated
 305 likelihoods and uncertainties in brackets.

306 *Table 4 Summary of risk estimates for pathway 2: 'Introduction through feed contaminated with viable Anisakis spp.*
 307 *larvae' (H=High, M=Medium, L=low, VL=Very Low, EL= Extremely Low N=Negligible).*

Step	Description	Likelihood (conditional)	Uncertainty
Release assessment			
1	Intermediate or paratenic hosts are used as raw material for the feed	Certain event	
2	Hosts used as raw material for the feed are infected	H	L
3	The parasite survive the treatment in feed mill	N (N)	L (L)
Exposure assessment			
4	Survived larvae are ingested	//	//
5	The parasite is not removed during the process of threading	//	//

308

309 *Table 5 Summary of risk estimates for pathway 3: 'Introduction through wild salmon that have accidentally entered the*
 310 *floating cages' (H=High, M=Medium, L=low, VL=Very Low, EL= Extremely Low N=Negligible)*

Step	Description	Likelihood (conditional)	Uncertainty
Release assessment			
1	Wild infected salmons are present in the area surrounding the farm	L	M
Exposure assessment			
2	Infected wild salmon enter the floating cage	N (N)	L (L)
3	The parasite is not removed during the process of threading	//	//

311

312

313

314 Table 6 Summary of risk estimates for pathway 4: 'Introduction through escaped salmon that have been infected offshore
 315 re-entering the cages.' (H=High, M=Medium, L=low, VL=Very Low, EL= Extremely Low N=Negligible)

Step	Description	Likelihood (conditional)	Uncertainty
Release assessment			
1	Escape of farmed salmons	L	L
2	Escaped salmons prey infected hosts	L (H)	L (L)
Exposure assessment			
3	Escaped salmons get infected and re-enter the floating cages	N (N)	L (L)
4	The parasite is not removed during the process of threading	//	//

316

317 Table 7 Summary of risk estimates for pathway 5: 'Introduction through the ingestion of infected hosts' (H=High,
 318 M=Medium, L=low, VL=Very Low, EL= Extremely Low N=Negligible).

Step	Description	Likelihood (conditional)	Uncertainty
Release assessment			
1	Infected hosts are present in the area surrounding the farm	H	L
2	The infected hosts penetrate the floating cages.	L (M)	H (H)
Exposure and establishment assessment			
3	Infected hosts in floating cages are eaten by high quality harvested salmons	VL (L)	H (H)
4	The parasite is not removed during the process of threading	VL (H)	H (L)

319 In pathway 2, having assessed the likelihood for step 3 as **Negligible** with **Low** uncertainty, the assessment of the
 320 cumulative likelihood did not continued beyond that stage and for the same reason, the assessment along pathways 3
 321 and 4 did not continue beyond steps 2 and 3 respectively.

322 4. DISCUSSION

323 In our study, the estimated cumulative likelihoods defined the risk of introduction of *Anisakis spp.* into Atlantic salmon
 324 farms (and commercialization of infected products) as *Negligible* or *Very Low* depending on the considered pathway. Our

325 formal qualitative estimations agreed with the available scientific evidence (Angot & Brasseur, 1993; EFSA, 2010;
326 Lunestad, 2003; Skov, Kania, Olsen, Lauridsen, & Buchmann, 2009; Wootten et al., 2010) who generally considered the
327 presence of vital anisakid larvae in farmed salmon as a very unlikely event.

328 With respect to the second pathway, our estimation coincides with the conclusions reported by EFSA, (EFSA, 2010) and
329 the outcome was characterized by a Low level of uncertainty, indicating strong evidence in support of the result. In fact,
330 to date, there are no evidence or reported cases indicating that nematodes of genus *Anisakis spp.* are able to survive the
331 processes at which the raw materials are subjected (Angot & Brasseur, 1993; Bristow & Berland, 1991; Inoue, Oshima,
332 Hirata, & Kimura, 2000; Lunestad, 2003). Recently, some proteins attributable to *Anisakis simplex* have been found in
333 processed fish products (Fæste, Plassen, Løvberg, Moen, & Egaas, 2014), but the risk related to allergic reactions due to
334 the presence of heat-resistant proteins (Alvaro Daschner, Cuéllar, Sánchez-Pastor, Pascual, & Martín-Esteban, 2002;
335 Pravettoni, Primavesi, & Piantanida, 2012), was beyond the scope of this study.

336 The cumulative likelihood obtained for the pathway 3 and 4 led to a *Negligible* risk of introduction and this would not
337 change even if further evidence led to the revision of the likelihood estimation for the first step of pathway 3,
338 (characterized by *Medium* uncertainty), to *High*.

339 The cumulative likelihood of the pathway 5 was considered to be *Very Low* but this outcome was characterized by *High*
340 uncertainty.

341 The route of introduction by ingestion of infected hosts, although characterized by high uncertainty, was the only pathway
342 leading to an overall estimation of the risk as greater than 'Negligible' and our formal findings seem to support the
343 hypothesis of the authors who recovered larvae of *Anisakis simplex* from farmed salmons (Marty, 2008; Mo et al., 2014).
344 Although characterized by a high level of uncertainty (because of the uncertainty in steps 2 and 3), the lack of evidence
345 found in the literature in support of this route is in agreement with our overall estimate, consequently, it can be
346 hypothesized that the high uncertainties in steps 2 and 3 are the result of lack of data and that it is unlikely that if further
347 data become available the estimation would move upward (i.e. greater than 'Very Low').

348 On the basis of the current knowledge of the biology of the system, and the typical practices adopted in the Atlantic
349 salmon farming, the overall risk of commercialization of product infested by viable larvae appears to be very low despite
350 the overall conservative approach adopted.

351

352 **Main assumptions and limitations.**

353 As outlined by the hazard identification, the assessment was made considering nematodes belonging to the genus
354 *Anisakis* without distinguishing between the different species, thus, similar properties amongst the species of genus
355 *Anisakis* were assumed. Moreover, it should be noted that because of the differences in the typical habitat between
356 *Anisakis spp.* and *Pseudoterranova spp.*, together with the likelihood of parasite's migration from the intestine to the
357 flesh being considered cautiously high with low uncertainty (making the different migration behaviour of the species no
358 more significant), the results obtained for *Anisakis spp.* could be reasonably extended to *Pseudoterranova spp.*

359 As infected salmon do not die or appear as unhealthy/unfit for human consumption, in the exposure assessment it was
360 assumed that in the processing line at farm level infected salmon are not recognized.

361 In the study, only plausible routes of introduction were considered in the release and exposure assessment; nevertheless,
362 since science cannot prove that a particular pathway does not exist there will always be a degree of uncertainty.

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