Sample size planning and the statistical significance of official controls by sampling

Cesare Ciccarelli,[1] Angela Marisa Semeraro,[1] Melina Leinoudi,[2] Vittoria Di Trani,[1] Anita Ciampana,[3] Elena Ciccarelli[4]

[1]*Local Public Health Service - Ascoli Piceno, San Benedetto del Tronto, Italy;* [2]*Chemist, Greece;* [3]*Veterinarian, Italy;* [4]*Biologist, United Kingdom*

Abstract

Acceptance sampling is important for food safety and is a relevant tool at production and official control levels, as it helps decision-making processes and verifies quality and food safety management. Generally, sampling plans are hypothesis tests of products that have been submitted for official appraisal and subsequent acceptance or rejection. The sample size is related to the set level of risk, the acceptable precision, and the tolerable misstatement size; therefore, sample size determination has a crucial role in setting up the accepted level of non-compliance and level of error. Using a simple predictive model based on combinatorics, this study showcases how sample size management can change the probability of rejecting good lots and/or accepting bad lots when the acceptance number is 0 (c=0). We showed that when c=0, a very high level of significance of the test corresponds to the high probability of rejecting a lot with an acceptable prevalence of defective items (type II error). We produced tables about the minimum sample size at different significance levels, which can be useful in the field. A paradigmatic example of the role of sample size in the acceptance-sampling plan is represented by the visual inspection for the detection of *Anisakid* larvae in fishery products. This study investigated this aspect and mainly referred to studies on the prevalence of larvae in farmed fish. We showed that, for lots ≥1000 items, the sample size is not strictly related to the lot size, but to draw a consistent control plan and reduce the variability in the clinical judgment, control authorities require a standardized approach. Because of this, the results on the prevalence of *Anisakid* larvae in farmed fish, if only based on sampling control plans, do not support a negligible risk statement, despite the claims reported in the EFSA opinion and several other studies.

Correspondence: Cesare Ciccarelli, Local Public Health Service - Ascoli Piceno, 63074 San Benedetto del Tronto, Italy.
Tel: +39.0735.7937474.
E-mail: cesare.ciccarelli@sanita.marche.it

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Introduction

Inspections on the food of animal origin are necessarily performed only on samples of the populations, called lots, and acceptance sampling is a relevant tool for food safety, especially for verification at production and official control levels, as it helps the decision-making processes and the achievement of quality and food safety management. Feasible inspections must be balanced between their effectiveness and their unavoidable margin of error, and related to their level of significance and the acceptable prevalence of defective items. In this case, it is crucial to ensure that the concerning sampling plan, as a statistic-based inspection procedure, is carried out on samples as representative as possible.

From a general point of view, sampling plans are hypothesis tests of products that have been submitted for official appraisal and subsequent acceptance or rejection. The decision is based on the pre-specified criteria and depends on the number of defective units found in the sample. Accepting or rejecting a lot is analogous to rejecting or not the null hypothesis in a hypothesis test (Dumicic *et al.*, 2006).

Since acceptance sampling is based on probability, the risk of making a wrong decision can be quantified and arises as a critical concern too. According to the Codex Alimentarius, a sampling plan takes into consideration the sample size, the inspection procedure, and the acceptance number (c) of defective items that represent the criteria for accepting or rejecting the tested lots (FAO/WHO, 2004).

The sample size is related to the set level of risk, the acceptable precision, and the size of tolerable misstatement; therefore, sample size determination has a crucial role and influences the accepted level of non-compliance and the level of error.

A paradigmatic example of the role of sample size in the acceptance sampling plan is represented by the visual inspection for the detection of *Anisakid* larvae in fishery products. The presence in finfish and cephalopods of ascaroid nematodes belonging to the

families *Anisakidae* and *Raphidascarididae* (Ángeles-Hernández *et al.*, 2020), usually called *Anisakids*, represents a relevant public health issue due to their zoonotic potential (Robertson, 2018). Human infection is associated with the ingestion of raw or undercooked seafood hosting viable third-stage larvae (L3) belonging to the *Anisakidae* family (Bao *et al*., 2017; Mattiucci *et al*., 2018). In addition to health implications, the presence of visible parasites in the flesh affects the quality, making fishery products repugnant to the consumer and reducing their commercial value.

In fact, this is a concern for food business operators (FBOs), official control authorities (CA) and consumers, and a large number of provisions have been issued at the European Union level.

Considering that no sea fishing grounds can be considered free from *Anisakids* and that aquaculture products can also be affected (EFSA, 2010; Ángeles-Hernández *et al*., 2020), the best prevention system is the combined application of freezing or heat treatments and an effective control system by trained FBOs, as stated in Regulation 853/2004 (European Commission, 2004).

According to Regulation 2074/2005 (European Commission, 2005), the visual inspection has to be performed on a representative number of samples. In particular, the FBOs "shall determine the scale and the frequency of inspection by reference to the type of fishery products, their geographical origin and their use" (European Commission, 2005).

The goal of this control is to ensure that the fishery products placed on the market for human consumption are not "obviously contaminated with parasites" (European Commission, 2004).

As stated in Regulation 2019/627 (European Commission, 2019), CA shall undertake risk-based tests to verify compliance with Regulation 853/2004 and Regulation 2074/2005.

Regulation 853/2004 stated that FBOs "must not place fishery products that are obviously contaminated with parasites on the market for human consumption" (European Commission, 2004 - ANNEX III, Section VIII, Chapter V letter D). Moreover, the freezing treatments do not need to be carried out if *inter alia* the FBO "verifies through procedures, approved by the Competent Authority, that the fishery products do not represent a health hazard with regard to the presence of viable parasites" (European Commission, 2004 - Chapter III letter D par. 3).

Several studies, based on sampling control plans, reported a negligible risk for farmed fish (Lunestad, 2003; Skov *et al*., 2009; EFSA, 2010; Wooten *et al*., 2010; Unger and Palm, 2016; Fioravanti *et al*., 2021; Karami *et al*., 2022), but the presence of parasites cannot be excluded (Marty, 2008; Mo *et al*., 2014; Cammilleri *et al*., 2018; Mercken *et al*., 2020; López-Verdejo *et al*., 2022); in these cases, controls aim to ensure the lower level of presence of parasites (very close to 0), and the role of the sample size arises as a crucial concern.

Moreover, ANNEX II, Section I, Chapter II of Regulation 2074/2005 states that "visual inspection shall be performed on a representative number of samples" and in the case of manual evisceration "in a continuous manner by the handler at the time of the evisceration and washing", whereas in case of mechanical evisceration "by sampling carried out on a representative number of sample being not less than 10 fish per batch" (European Commission, 2005). For fish fillets or fish slices, when an individual examination is not possible "a sampling plan must be drawn up and kept available for the Competent Authority" (European Commission, 2005).

Therefore, controls in this field, including official ones, must be based on risk analysis and performed, by visual inspection of representative samples. This control aims to verify the absence of parasites (acceptance number: c=0); and the sample size is crucial to ensure precision and consistency of the results. The concerning decisions, as clinical judgments, can be affected by a relevant level of variability (Kahneman *et al*., 2021). However, the European Union has not yet issued guidelines in this field aimed, for instance, at setting uniform criteria to define the sampling size and to determine the batches' acceptability. Using a simple predictive model based on combinatorics, this study aims to investigate how the management of sample size can change the probability of rejecting good lots and accepting bad lots when the acceptance number is zero (c=0). Consequently, it highlights how the sample size setting affects the consistency of the controls, whether performed by FBOs or CAs.

For this purpose and as a paradigmatic example, the study assumes the inspection for the detection of *Anisakid* larvae in fishery products, and mainly refers to studies on the prevalence of larvae in farmed fish exempted from freezing treatments, given the crucial impact of this exemption on food safety.

Materials and Methods

An acceptance single sampling plan is a decision rule used to accept or reject a lot, based on the inspection results of one random sample taken from the lot. Since the entire lot is not inspected, sampling will always incur a certain risk, as only the sample is known. This leads to the risk of making two types of errors in the accept/not accept decision. From the point of view of the hypothesis test, these errors are i) rejecting a lot that should be accepted (reject $H_0|H_0$ is true): this is called type I error and the probability P=α is called significance; ii) accepting a lot that should be rejected (not reject $H_0|H_0$ is false): this is called type II error and the probability is P=β. The protection level of the test is P=1-α and the power of the test is P=1-β.

Since the probability of a type I error (α) has an inverse relationship with the probability of a type II error (β) and β is the complement of the power of the test, an increase in the value of α determines an increase in power, and in sample size. For the study of this relationship in the condition of acceptance number c=0, we used a predictive model based on combinatorics to determine the probability of obtaining a sample without defective items, given the lot size, the assumed prevalence of defective items and the sample size.

Theoretically, the observed population of the lot could be represented as a set *F*, where $F \subseteq N$, consisting of two subsets *Fn*, the good items, and *Fp*, the defective items, such that $Fn \subseteq F$, $Fp \subseteq F$, $Fn \cap Fp = \phi$ and $Fn \cup Fp = F$.

Along this line, a random sample of that population could be represented as a set *S*, where $S \in N$, consisting of two subsets *Sn*, the good items in the sample, and *Sp*, the defective items, such that $Sn \subseteq Fn$, $Sp \subseteq Fp$, $Sn \cap Sp = \phi$, and $Sn \cup Sp = F$.

Furthermore, the assumed prevalence of defective items is $\nu_p = Fp/F$ and that of good items is $\nu_n = Fn/F$ such as $\nu_p + \nu_n = 1$.

Since each inspected item could be either defective or not and assuming a correct random sampling, we calculated the probability of extracting a sample of only good items, starting from the size of the observed lot and the number of defective items we assumed to be present.

We followed a similar approach to identify the minimum sample size to obtain a significant result once the lot size and the maximum acceptable prevalence of defective items were known. In addition, we studied a specific condition of the acceptance sampling plans, when the rate of defective items has to be very close to 0 and $\nu_p \approx 0$.

We also used this predictive model to study the performances of different sampling plans for visual inspection for the detection of *Anisakid* larvae in fishery products. We studied the performances of three sample sizes made of 10, 28 and 40 items, respectively.

The first one is based on the minimum sample size stated in Regulation 2074/2005 about the controls on mechanical eviscerated fisheries, the unique case of sample size exactly stated by EU Regulations (European Commission, 2005); the second and the third ones are the values most often implemented for official controls in Italy for lots ≤1500 kg and >1500 kg respectively. We made a graphic, which we called sampling operating characteristic (SOC) curve, to display the performance of a given sampling plan by plotting the lot probability acceptance *versus* a range of proportions of defective items. Taking into account the studies on the prevalence of larvae in farmed fish, we rallied the available bibliography on this topic in Table 1 (Marty, 2008; Skov *et al.,* 2009; Wooten *et al.*, 2010; Levsen and Maage, 2016; Unger and Palm, 2016; Cammilleri *et al*., 2018; Fioravanti *et al.*, 2021; Karami *et al.*, 2022). Assuming those results were effective to certify the exemption from freezing treatments and when data on the size of the studied population were available, we calculated the rate of fish with at least one vital parasite, that would theoretically be put on the market, based on the stated sample size and the related maximum prevalence of parasitized subjects at a significance level of 95%. All calculations were performed using Libre Office Calc. Version 6.4.4.2 spreadsheet (https://it.libreoffice. org/download/download/).

Results and Discussion

This study investigated the relationship between sample size and the other variables of the sampling plan, such as the maximum acceptable prevalence of defective items, significance, and power of the test, taking into account acceptance sampling as a hypothesis test and the condition of acceptance number c=0. For a single sampling plan, since each item inspected is either defective or not and, assuming that a correct random sampling is carried out, starting from the size of the observed lot and the maximum acceptable number of defective items, we can calculate the probability of extracting a sample constituted by good items only.

This probability is expressed in Equation 1:

$$P_{(Sp=\emptyset|F,Fp,S)} = \prod_{i=1}^{S} \frac{Fn}{F} \times \frac{Fn-1}{F-1} \dots \frac{Fn-S}{F-S} = \frac{\binom{Fn}{S}}{\binom{F}{S}} = \frac{Fn!\,(F-S)!}{F!(Fn-S)!} \quad \text{[Eq. 1]}$$

For the hypothesis test $H_0: v_p \geq v_0$, where v_0 is the maximum acceptable number of defective items, we know that $P(Sp=\phi|F, Fp, S)$=a. This probability value α is related to 3 variables - the lot size F, the maximum acceptable number of defective items Fp and the sample size S - and weighs the ability to recognize that the prevalence of defective items is not higher than assumed. It is a p value and represents the statistical significance that helps quantify whether the obtained result is likely due to chance or some factor of interest; it is also the probability of type I error. Furthermore, the probability of extracting a sample with at least one defective item despite $v_p < v_0$ can be expressed as in Equation 2:

$$P_{(Sp\neq\emptyset|F,Fp-1,S)} = 1 - \prod_{i=1}^{S} \frac{Fn+1}{F} \times \frac{Fn}{F-1} \dots \frac{Fn+1-S}{F-S} = 1 - \frac{\binom{Fn+1}{S}}{\binom{F}{S}} = 1 - \frac{(Fn+1)!\,(F-S)!}{F!(Fn+1-S)!} = \beta \quad \text{[Eq. 2]}$$

The value β is the probability of occurrence of the type II error, in other words, the probability of not rejecting a false hypothesis.

Accordingly, the probability of obtaining a sample with at least one defective item could be expressed as in Equation 3:

$$P(Sp\ \emptyset|F, Fp, S)=1-P(Sp=\emptyset|F, Fp, S). \quad \text{[Eq. 3]}$$

And since $P(Sp\neq\phi|F, Fp, S) \approx P(Sp\neq\phi|F, Fp-1, S)$, $1-\alpha\approx\beta$. Therefore, when c=0 the protection level of the test is $P=1-\alpha\approx\beta$ and the power of the test is $P=1-\beta\approx\alpha$.

Starting from Ángeles-Hernández *et al.* (2020), we created the SOC curve. This graph depicts the lot acceptance probability on the vertical axis, and on the abscissa, the defective items are expressed in terms of percentage which allows us to pinpoint the couple pair of values: maximum acceptable number of defective items and associated probability. Figures 1 and 2 show some examples of SOC curves for different sample sizes when the lot is made of 10^3 or 10^6 items. We followed a similar approach to that of Ángeles-Hernández *et al.* (2020) to identify the minimum sample size to obtain a

Table 1. Available studies on the prevalence of larvae in farmed fish and theoretical rate of parasitized fish put on the market (assuming that results are effective to certify the exemption from freezing treatments and based on the lot size, sample size, and the maximum prevalence of parasitized subject, at a significance level of 95%).

Authors	Species	Geographical area	Subject matter	Sample-size	Estimated farmed fish population	Maximum prevalence of parasitized subject (%)	Parasitized subjects put on the market
Karami *et al.*, 2022	*O. mykiss*	Denmark	Sea cage	170	Data not available		
Levsen and Maage, 2016	*S. salar* *S. salar (grunts)*	Norway	Sea cage Sea cage	3525 659	230×106	0.11	2.5×105
Unger and Palm, 2016	*O. mykiss*	Germany	Sea cage	105	Data not available		
Skov *et al.*, 2009	*O. mykiss*	Denmark	Sea cage	166	Data not available		
Cammilleri *et al.*, 2018	*D. labrax*	Italy	Market	151	Data not available		
Marty, 2008	*S. salar*	Italy	Sea cage	894	Data not available		
Fioravanti *et al.*, 2021	*S. aurata* *D. labrax*	Italy Spain Greece Italy Spain Greece	Sea cage Sea cage	2753 2761	3×108 3×108	0.12 0.29	3.6×105 8.7×105
Wooten *et al.*, 2010	*S. salar*	United Kingdom	Sea cage	720	38×106	0.42	1.6×105

O. mykiss, Oncorhynchus mykiss; S. salar, Salmo salar; D. labrax, Dicentrarchus labrax; S. aurata, Sparus aurata.

significant result, once known the lot size and assumed the maximum acceptable prevalence of defective items.

We calculated the minimum sample size *S*, which would allow us to test for rejection of the null hypothesis $H_0: v_p \geq v_0$ using the system of inequalities of formula 4:

$$\begin{cases} \frac{Fn!\,(F-S)!}{F!(Fn-S)!} < \alpha \\ \frac{Fn!\,(F-S+1)!}{F!(Fn-S+1)!} \geq \alpha \end{cases} \quad \text{[Eq. 4]}$$

Taking into account different lot sizes, from 10^2 to 10^6, and for a wide range of v values, from 0.005 to 0.25, we used formulas to calculate the lowest sample sizeable to obtain a significant result (Crotta *et al.*, 2016).

Tables 2-4 resumed those sample-size values at three different levels of significance: 0.05, 0.01 and 0.001, respectively. They could help the inspectors on the field to make the correct decision about sample size. Moreover, we studied the relationship between sample size and significance of a sampling plan at a specific condition, when the rate of defective items is very close to 0. In that case, when $v_p \approx 0$, the relative sample size S must satisfy $F(1-\alpha) < S$ to reject the null hypothesis $H_0: F_p \approx 1$, since $Fn \approx F-1$.

It is clear that, in this specific condition, to obtain an accurate statistical significance, the test p value needs to be low, at least <0.05, and the sample size *S* has to reach values close to the population size *F*, making sampling virtually impossible.

The performance of visual inspection for the detection of *Anisakid* larvae in fishery products has been studied using the SOC curve shown in Figure 3.

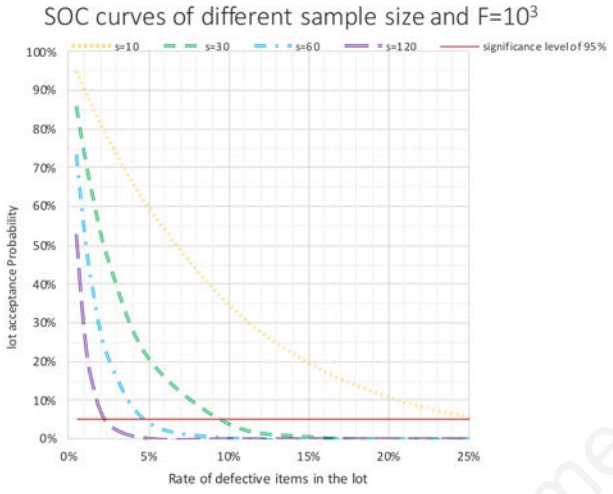

Figure 1. Sampling operating characteristic (SOC) curves of different sample sizes and a lot of 103 items displaying a comparison of lot probability acceptance *versus* a range of proportions of defective items, taking into account different sample sizes.

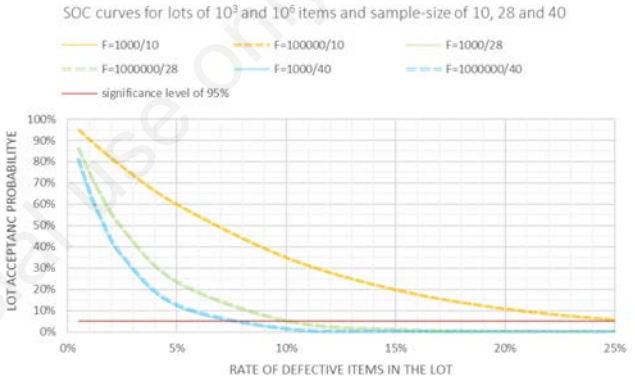

Figure 3. Sampling operating characteristic (SOC) curves for sample sizes of 10, 28, and 40 with lot sizes of 10^3 and 10^6 items and displaying a comparison of lot probability acceptance *versus* a range of proportions of defective items, taking into account different sample sizes.

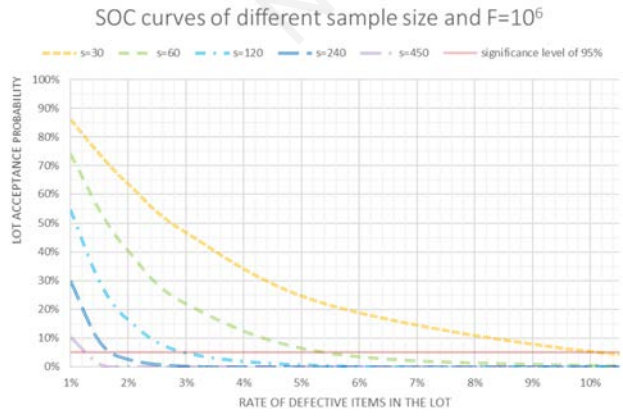

Figure 2. Sampling operating characteristic (SOC) curves of different sample sizes and a lot of 10^6 items displaying a comparison of lot probability acceptance *versus* a range of proportions of defective items, taking into account different sample sizes.

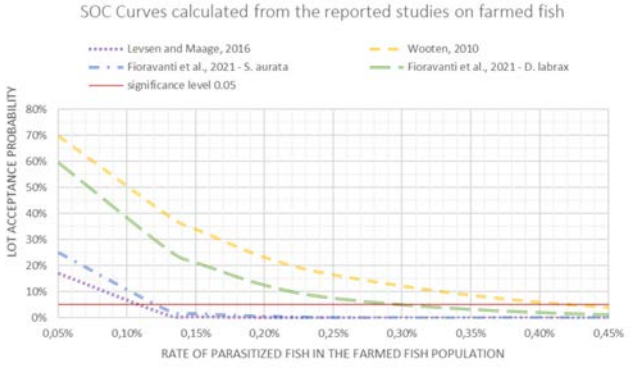

Figure 4. Sampling operating characteristic (SOC) curves related to the reported studies on the prevalence of *Anisakid* larvae in farmed fish items and displaying a comparison of lot probability acceptance versus a range of proportions of defective items, taking into account different sample sizes. *D. labrax, Dicentrarchus labrax; S. aurata, Sparus aurata.*

Table 2. Minimum sample size values to obtain a significance level α=0.05 in relation to the lot size, the maximum acceptable prevalence of defective items, and c=0.

α=0.05	Maximum acceptable prevalence of defective items								
	0.005	0.010	0.015	0.025	0.050	0.100	0.150	0.200	0.250
lot size									
100	96	96	78	63	45	25	17	13	10
150	143	117	95	79	46	26	17	13	10
250	194	158	131	86	51	27	18	14	11
500	316	225	156	102	56	28	19	14	11
750	395	234	165	109	56	28	19	14	11
1000	450	258	180	112	57	29	19	14	11
1500	468	271	182	113	58	29	19	14	11
2500	514	281	189	115	58	29	19	14	11
5000	564	290	195	117	59	29	19	14	11
10,000	581	294	197	118	59	29	19	14	11
15,000	586	296	197	118	59	29	19	14	11
100,000	596	298	199	119	59	29	19	14	11
1,000,000	598	299	199	119	59	29	19	14	11
1,000,000	598	299	199	119	59	29	19	14	11

Table 3. Minimum sample size values to obtain a significance level α=0.01 in relation to the lot size, the maximum acceptable prevalence of defective items, and c=0.

α=0.05	Maximum acceptable prevalence of defective items								
	0.005	0.010	0.015	0.025	0.050	0.100	0.150	0.200	0.250
lot size									
100	100	100	90	78	59	36	25	19	15
150	149	135	117	102	65	38	26	20	15
250	225	196	170	120	73	41	27	20	16
500	392	300	211	148	83	42	28	21	16
750	512	327	238	160	84	43	28	21	16
1000	601	368	263	167	86	43	28	21	16
1500	655	395	271	170	88	44	29	21	16
2500	744	419	284	175	89	44	29	21	16
5000	840	438	296	179	89	44	29	21	16
10,000	878	448	301	181	90	44	29	21	16
15,000	892	452	302	181	90	44	29	21	16
100,000	915	458	305	182	90	44	29	21	17
1,000,000	919	459	305	182	90	44	29	21	17

Table 4. Minimum sample size values to obtain a significance level α=0.001 in relation to the lot size, the maximum acceptable prevalence of defective items, and c=0.

α=0.05	Maximum acceptable prevalence of defective items								
	0.005	0.010	0.015	0.025	0.050	0.100	0.150	0.200	0.250
100	100	100	97	90	74	48	35	27	22
150	150	145	135	123	85	53	36	28	22
250	242	225	205	155	101	58	39	29	23
500	450	373	288	204	118	62	41	30	24
750	616	432	326	226	122	63	42	31	24
1000	748	497	367	239	126	64	42	31	24
1500	866	552	387	247	129	65	42	31	24
2500	1029	601	413	257	132	65	43	31	24
5000	1123	643	437	266	133	66	43	31	24
10,000	1288	665	447	270	134	66	43	31	24
15,000	1317	672	451	271	135	66	43	31	24
100,000	1369	686	457	273	135	66	43	31	25
1,000,000	1378	688	457	273	135	66	43	31	25

It is easy to recognize that: i) the sample size is inversely proportional to the assumed prevalence of parasitized subjects and directly related to the power of the test; ii) the sample size is not relevant when lots are ≥1000 items; indeed, the SOC curves of different sample size are superimposable, so increasing the sample size improves the power of the sampling but it is not related to the lot size; iii) for the examples taken into account, the implicitly assumed prevalence of parasitized subjects is not entirely negligible and, with larger sample size, rise to the level of 7.5% with a significance of 95%; iv) to achieve a consistent network of controls reducing the variability of related clinical judgment, measures of decision hygiene are needed, such as standardized instructions or guidelines (Kahneman *et al.*, 2021).

The predictive model was applied to the studies on the prevalence of *Anisakid* larvae in farmed fish, as shown in Table 1. Based on the sample size reported in each study and the size of the population taken into account, when available, we calculated the maximum prevalence of parasitized fish at a significance level of 0.05 and, accordingly, the number of fish with at least one vital parasite theoretically put on the market, assuming an exemption from the freezing treatment. The related SOC curves are shown in Figure 4, whereas the number of parasitized fish hypothetically put on the market is reported in Table 1. The SOC curves show how, for some studies, the performances of those sampling plans are not very efficient and the rate of implicitly accepted parasitized fish could be relevant.

It is clear that, despite the statements of those studies and the very high number of farmed fish, the number of those hypothetically parasitized and put on the market does not emerge as negligible and the exemption from the freezing treatment needs to be supported by collateral studies, such as qualitative assessment of the probability of the introduction of alive parasites in mariculture farms (Crotta *et al.*, 2016).

Conclusions

In the food safety quality control fields, inspection based on acceptance sampling is a clinical judgment concerned with the decision to accept or reject a lot (or batch) of goods.

Feasible inspections have to be balanced between effectiveness and unavoidable margin of error, related to the level of significance and the acceptable prevalence of defective items.

The design of this process includes decisions about sampling *versus* complete inspection, the maximum acceptable prevalence of defective items, the significance of the test (α), the power of the test (1-β) and sample size.

This study investigated the relationship between those variables when the acceptance number of defective items in the sample is zero (c=0), a frequent situation in the field of food safety.

The resulting predictive model, based on combinatorics, showed that, when c=0, there is a very close relationship between α and β, such that α≈1-β, so a very high level of significance of the test corresponds to a high probability of rejecting a lot with an acceptable prevalence of defective items (type II error). Tables 2-4 about the minimum sample size at the related significance level could be useful in the field. This predictive model applied to the inspection for detection of *Anisakid* larvae in fishery products showed that, in the case of the visual inspection, starting from lots bigger than 1000 items, the sample size is strictly related to the exactitude - as the power of the test - of the results and it is not related to the lot size; however, to implement a consistent network of controls and to reduce the variability in this kind of clinical judgment, it needs a uniform approach to set the sample size. Moreover, despite what is stated in the EFSA opinion, in the case of the studies on the prevalence of *Anisakid* larvae in farmed fish only based on sampling plans, the results do not appear to be satisfactory, and the statement of negligible risk needs to be supported by collateral studies.

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