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Evaluating the performance of sampling plans for phycotoxins in shellfish: Improvement of an existing method

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ABSTRACT

According to the EU Regulation 854/2004, sampling plans must be set up to monitor production areas for the level of okadaic acid (OA) equivalents in live mussel. The level of these toxins, which are produced by harmful algal blooms, must not exceed 160 μg/kg of raw meat (Regulation 853/2004/EC). A sampling plan assessment consists in obtaining an OC (Operating Characteristic) curve showing both consumer and producer risks. The first risk is the risk of opening a shellfish area for harvest while the contamination level is above the threshold; whereas the second risk is the risk of closing a shellfish area having a contamination level under the threshold.

For sampling plan validation purposes, a classical mathematical method was improved for the prediction of variance as function of the mean contamination level thanks to prior knowledge of the theoretical distribution fitting the observed OA levels among individual mussels. Indeed, knowing that, thanks to a regression analysis of literature data, for the lognormal distribution the scale parameter was observed to be directly proportional to the location parameter, the regression bias could be lowered. Literature data from Norway and Sweden showed different levels of variability between contamination events and depuration. However, the highest variability level was chosen to propose a best fit sampling plan in order to have a better approach of reality. It consisted of taking two samples of 50 mussels (*Mytilus* sp.) for this geographic location (Norway and Sweden).

1. Introduction

Okadaic acid (OA) and its analogues, the dinophysis toxins (DTX-1, DTX-2), form together the group of OA-toxins. They are produced by toxic dinoflagellates, which are part of the phytoplankton. As bivalve shellfish feed on phytoplankton, they become contaminated by the presence of these toxic dinoflagellates in seawater and accumulate the toxins, which in turn become available to both animal and human consumers. The mussel *Mytilus edulis* accumulates the toxin in its digestive gland (Pillet et al., 1995). Rossignoli et al. (2011) reported that even when free OA was found in considerable amounts in the digestive gland of mussels, in faeces, nearly all the detected OA was found in the esterified form, thus suggesting that acylation could be an important step in the depuration of DSP toxins from the bivalves. For this reason, the amount of esterified toxins in hepatopancreas can be determined, together with the amount of free toxins (Duinker et al., 2007). It was found esterified toxins are cleared faster than free toxins, either in wild populations or in laboratory tanks (Vale, 2004, 2006). In *Mytilus* spp., OA and DTX2 are not completely esterified, thus remaining a large proportion of free toxins that in turn depurate slowly. The biotransformation in this bivalve is also toxin-specific: OA is commonly more esterified than DTX2 (Vale et al., 2008). In the same way, Torgersen et al. (2008) found that in mussels a higher proportion of OA was esterified compared to DTX1 and DTX2 and the esters of DTX1 depurated significantly slower from mussels compared to esters of OA and DTX2. Furthermore, there is a high contamination level variability between individual mussels, even for the ones taken at the same sampling place and at the same time. Due to water currents and variability of food accessibility along the water column, as well as between individuals located close to the rope or far from it, there is an inter-individual variability during a contamination event. Concerning depuration variability, Haamer et al. (1990) and Sampayo et al. (1990) reported that depuration is increased in case of non-toxic food availability. However, Svensson and Förlin (2004) did not found a clear relationship between depuration rates and presence or absence of non-toxic food. Depuration variability in the same environmental conditions might be explained by varying biotransformation rates between individuals. This inter-individual

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variability has got an impact on the accuracy of the contamination level that can be found in a global sample. Indeed, due to their threat for human health, the level of OA in mussels must be monitored in order to decide of the status of a growing area and it must not exceed 160 μg/kg of raw meat (Regulation 853/2004/EC). For this reason, a sampling plan assessment must be set up in order to decide of the appropriate sampling plan, and more precisely of the relevant number of samples and of the sample size. Furthermore, a variability study might enable to give some clues and hypothesis about varying accumulation/depuration rates, thereby being a useful supplementary material to accumulation/depuration studies. The existing reference method for the mathematical validation of sampling plans was improved. The lognormal distribution was fitted to the observed OA equivalent levels among individual mussels in a previous work (Wesolek et al., 2014). This previous knowledge was incorporated in a regression analysis, considering that, the scale parameter was directly proportional to the location parameter thanks to empirical results. By this process, the regression bias could be lowered. Then, the probabilities of acceptance, as determined by different sampling schemes, were plotted against mean OA lot concentrations. The curves obtained, which are referred to as Operating Characteristic (OC) curves, enable one to quantify consumer and producer risks. Consumer risk is the probability that a lot having a true concentration above the threshold (unsafe lot) is authorized for sale and consumption. Producer risk is the probability that a lot at a true concentration lower than the threshold (good lot) is rejected for sale. Then a best fit sampling plan could be proposed, taking into account the two risk types, as well as considering the practical feasibility of the sampling plan.

2. Materials and methods

On a general basis, the sampling plan validation method always requires a prior knowledge of the distribution that fits the variable under study. Sampling plans have got a broad application in monitoring of process compliance with per example the proportion of defectives in an industrial process. In the food safety field, when contamination data are studied, concentration levels and standard deviation are used to compute the probabilities of acceptance. For chemical contaminants, Whitaker's method is the reference method, and it improves the sampling plan validation method in the way that it assesses the variance as function of the mean contamination level thanks to a regression equation (Whitaker et al., 1972, 2007a,b, per example). Indeed, variance is not a constant, as it increases along with mean contamination level. Furthermore, considering that variance components are independent, sampling, subsampling and analytical variance must be added to obtain total variance. For heterogeneously distributed contaminants, Whitaker and co-workers always found that the subsampling variance and analysis variance were negligible in comparison with total variance. So for this reason, the negligible variances were not calculated in this work. This is a slight modification of Whitaker's method that makes the method easier to undertake, with little loss of accuracy. We further improved Whitaker's method, considering that the link between variance and mean concentration can be better assessed by the distribution type rather than by strict classical regression. All these calculation steps are further explained in the following sections and used to evaluate different sampling strategies designed to detect potentially harmful levels of okadaic acid in mussels.

2.1. Theoretical distribution

To examine okadaic acid in mussels, we used, in previous work, the data of Dr. Arne Duinker who has supplied us (personal communication) with raw data on individual mussels contaminated with okadaic acid toxin equivalents, obtained during field experiments that lead to a publication (Duinker et al., 2007). These data consist of OA levels in mussels contaminated on collectors cultured at high density in a stratified fjord. Four different lots were sampled, and all the samples from a given lot were taken at the same sampling point, at the same time, knowing that each sampling point and sampling time was specific to each lot. For each lot: 29 or 30 samples were taken, each sample consisting of one mussel. Then each individual mussel was submitted to chemical analysis. Given the Regulation 853/2004/EC, the data, expressed in concentration in steamed mussels, must be converted to concentration in raw mussels. This conversion is done according to McCarron (McCarron et al., 2008). They published a theoretical conversion value: the concentration level in steamed meat must be divided by 1.2667 to obtain the concentration level in raw meat. The contamination levels were found to fit a lognormal distribution for each lot in previous work (Wesolek et al., 2014).

2.2. Variance and mean contamination data

2.2.1. Data used

Variance and mean concentration data were gathered from the literature. However, the publications selected were only the ones from Norway and Sweden. The reasons are the following: the climate and geographic location of these two countries are very similar; the mussel species is *Mytilus edulis* in both countries; the theoretical distribution has been previously assessed on data from Norway. More generally, the variability pattern is expected to be the same in both countries. Data were compiled from three publications (Duinker et al., 2007; Lindegarth et al., 2009; Svensson and Förlin, 2004) and one thesis (Wrange, 2008). These publications reported data on individual mussels or on pools of mussels. For individual mussels, at least five samples were reported for each standard deviation calculation, which is a sufficient number to ensure a correct representativeness. On the contrary, for pools of mussels, only samples consisting three mussels were considered, whereas samples of fifty mussels were not taken into account, due to a loss in representativeness. In some cases, published levels of OA concentration data were given for the hepatopancreas, and had to be re-calculated as whole flesh concentrations, because the European Regulation 853/2004 states that the okadaic acid concentration must be given per kg of whole flesh.

2.2.2. Variance for pools or individuals

If the difference in means is exclusively attributed to the sampling error, then the samples have to be randomly drawn from the same population. Two factors determine the magnitude of the sampling error: population variance, and the number of individuals in the sample size:

1. For population variance: the larger the population variance, the larger the sampling error.
2. For the number of individuals in each sample: the larger the number of individuals sampled, the smaller the sampling error. This principle is called the law of large numbers.

The last factor requires further explanation:

Variability between sub-samples consisting of pools of individuals is the variability between means. Indeed, we can consider that the OA concentration of a pool is equal to the mean of the concentrations of the individuals in the pool. The standard error of the mean is the standard deviation of the sample mean estimates of a population mean. It is usually estimated by the sample estimate of the population standard deviation divided by the