

TECHNICAL NOTE

Web-based application to guide sampling on salmon farms for more precise estimation of sea louse abundance

Jaewoon Jeong¹  | Crawford W. Revie^{1,2}

¹Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Charlottetown, Prince Edward Island, Canada

²Department of Computer and Information Sciences, University of Strathclyde, Glasgow, UK

Correspondence

Jaewoon Jeong
Email: jwjeong79@gmail.com

Funding information

FHF – Norwegian Seafood Research Fund, Grant/Award Number: FHF 901411; Ocean Frontier Institute

Abstract

Objective: Efficiently managing sea lice on salmon farms through active surveillance, crucial for lice abundance estimation, is challenging due to the need for effective sampling schemes. To address this, we developed an application that considers infestation levels, farm structure, and management protocols, enhancing the precision of sampling strategies for sea louse abundance estimation.

Methods: Simulation-based methods are valuable for estimating suitable sample sizes in complex studies where standard formulae are inadequate. We introduce *FishSampling*, an open Web-based application tailored to determine precise sample sizes for specific scenarios and objectives.

Result: The model incorporates factors such as sea lice abundance, farm pen numbers, potential clustering effects among these pens, and the desired confidence level. Simulation outcomes within this application provide practical advice on how to decide on the number of fish and pens to sample, under varying levels of assumed clustering.

Conclusion: This approach can be used across the salmon aquaculture sector to improve sampling strategies for sea lice abundance estimation and balance surveillance costs against health objectives.

KEYWORDS

salmon farm, sampling strategy, sea lice, surveillance systems, Web-based application

INTRODUCTION

Sea louse infestation remains one of the most serious challenges to salmon aquaculture in many regions. Infestation with this ectoparasite can pose a risk to salmon health and reduces fish growth, thus decreasing aquaculture productivity (Abolofia et al. 2017). It has also been suggested that such infestations may contribute to declines in wild salmonid populations (Krkosek et al. 2007; Rogers et al. 2013; Nekouei et al. 2018). To manage sea louse infestation on salmon farms, farm operators use a variety of methods (Overton

et al. 2019), including, among others, parasiticides to kill the parasites, fallow periods between production cycles where farms are emptied of fish to interrupt the parasite's life cycle, mechanical delousing to remove sea lice from salmon, and cleaner fish that feed on sea lice attached on salmon. In many instances, neighboring salmon farms adopt integrated pest management (IPM) to limit the exchange of sea lice among farms (Brooks 2009) and reduce external infestation pressure (McEwan et al. 2019). The success and maintenance of these interventions are contingent on an accurate and precise surveillance system.

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To be successful, a surveillance system must accurately estimate the abundance of sea lice, defined as the number of sea lice per fish, a standard metric used to measure levels of sea lice infestation, by sampling salmon. Liu and vanhauwaer Bjelland (2014) noted that the accuracy of sea lice abundance measurements were critical to effective resource management for salmon health. It is also obvious that errors in sea lice estimation may result in unnecessary interventions, with all of the cost and associated negative outcomes, or in the poorly timed application of required interventions. In addition, salmon-producing countries have regulations or guidelines in place that sea lice abundance should not exceed certain “treatment threshold” limits (Revie et al. 2009), with a range of negative consequences when such limits are breached. For these and other reasons, effective sea lice surveillance programs are critical, with appropriate sampling strategies being the cornerstone to such effectiveness.

Cost-efficient sampling strategies form the basis for effective surveillance in animal production systems (Alba et al. 2017). Due to the large number of fish on a salmon farm, the proportion sampled will always be small. The unnecessary handling of fish should also be avoided due to the potential stress that this may cause to the animals. It is therefore a requirement of responsible surveillance that a minimum sample size be determined, based on numbers that can generate an acceptable level of sampling precision. The impact of inaccurate estimates of sea lice abundance on lice management has been described using mathematical models (Jeong et al. 2021). In addition, the prevention of unnecessary treatment should help minimize the mortality associated with stress during such interventions (Overton et al. 2019). The optimal sampling strategy will be different, depending on farm structure, sea lice abundance, and the level of parasite clustering among pens. In addition, the desired level of precision will have an important bearing in determining a suitable sampling regime. This implies that we should not expect some absolute numbers that can be applied across all farms, or even to every case of sampling within a given farm.

The development of a practical method to simulate sampling events that accurately reflect sea lice abundance on salmon farms has significant benefits both in terms of cost savings and improved fish welfare. This study illustrates the use of *FishSampling*, a flexible and accessible modeling tool for salmon farm operators responsible for fish health on salmon farms, to estimate sea lice abundance and to support assessments as to when the treatment threshold may have been exceeded. We describe how the application was created and then, using some actual sampling protocols for different scenarios, illustrate how the application can be used in a field setting. While

Impact statement

The open-source application *FishSampling* enhances sea lice monitoring on salmon farms with a novel simulation-based approach for sample size determination. It offers precise estimates of sea lice abundance, crucial for regulatory purposes, and aids in the efficient allocation of sampling resources.

the tool is suitable for all species of sea louse (e.g., *Caligus rogercresseyi* in Chile, or the other *Caligus* spp. found elsewhere around the globe), the examples given will tend to be most closely aligned to sampling situation as they relate to salmon louse *Lepeophtheirus salmonis*, the species most commonly of concern on Atlantic Salmon *Salmo salar* farms outside of Chile.

METHODS: MODELING

We set up a hypothetical salmon farm consisting of multiple pens in which salmon can be infested with sea lice. Our model assigns a certain level of sea lice infestation for each fish on the farm based on the negative binomial distribution (Heuch et al. 2011). This distribution, which has a higher variance than its mean, as opposed to the Poisson distribution, where the mean and variance are equal, has been found to better approximate the nonrandom manner in which sea lice are observed on salmon in fish farms (Jeong and Revie 2020). The shape of a particular negative binomial distribution depends on two parameters: the mean (μ) and a dispersion parameter (κ). In our models, μ is the average number of sea lice per fish (i.e., abundance) while κ determines the shape of the distribution (i.e., variance). It was found that various levels of abundance across sites in several countries could be described by a reasonably narrow range of κ with a mean value of 2.19 (Jeong and Revie 2020). Once a user inputs an average abundance (μ) of sea lice in a farm, this abundance and the dispersion parameter of 2.19 (κ) are used to stochastically determine the number of sea lice present on each fish in the farm. These simulated fish are then randomly sampled from the farm using the Monte Carlo method, from which an observed mean abundance for the farm can be estimated.

We provide an option to consider the impact of different sea lice abundance levels being present among pens when estimating the lice abundance for the whole farm. The pattern of sea lice infestation often differs from pen to pen, and this clustering effect has been found to

significantly affect abundance estimation during sampling on salmon farms (Revie et al. 2007). The degree of clustering associated with sea lice infestation on fish among pens can be measured using the intraclass correlation coefficient (ICC), where a high ICC value signifies that the number of sea lice on fish resembles other fish in a given pen on a farm, as compared to infestation patterns over the whole farm. A high ICC is consistent with a situation where abundance levels differ markedly among pens on a given farm. In a study of sea lice abundance data from Scottish salmon farms, it was found that the ICC values typically ranged from 0 (no clustering) up to around 0.35 (Revie et al. 2005). In our simulations, probable ranges of ICC values are provided by the application, depending on the presumed abundance. Users can check how the simulated abundances of each pen are distributed based on the ICC value they specify. If the user wishes to assume that differences in abundance among pens on a farm is not considerable, they may omit any clustering effect (which also results in much shorter simulation run times).

The Web application includes three scenarios, where each scenario is designed to support sampling for a specific purpose. Thus, after choosing one of these three scenarios, the user is required to input specific conditions, such as sample size, assumed abundance, and number of pens. The number of fish in a pen was set as a reasonably small population size of 2000 in the interest of simulation efficiency. (In trials based on fish numbers well in excess of 2000 fish, it was found that little variability was generated in the sampling results.) By setting up different simulations based on these inputs, the application can generate feedback on the probability that the sampling purpose is likely to be satisfied. We used the R statistical environment to run the simulations (R Core Team 2022) and the *Shiny* package (Chang et al. 2017) to build the interactive Web application of *FishSampling*, which is freely available at <https://jaewoonjeong.shinyapps.io/FishSampling>. In addition, the source code is published on GitHub (<https://github.com/jaewoonjeong/FishSampling>).

RESULTS

Sampling Scenarios

Three scenarios were designed that satisfy different goals when sea lice abundance is being estimated through sampling (Figure 1). Scenario 1 provides the user with a probability that an estimated abundance will fall within the extent of two abundance values. Using scenario 1 provides an insight into the level of precision that can be expected given a certain sample size and cage selection choices. Scenario 2 provides a “special case” of this

range comparison by generating the probability of how likely it is that an estimated abundance will be higher than a specified level of abundance. This scenario has been designed to be used in the context of lice treatment thresholds, where it is important to determine whether the estimated abundance is lower than some regulated sea lice limit. Scenario 3 is designed for the case where the user wishes to compare two abundance levels, for example, subsequent to a sea lice treatment event. The output generates a probability as to how likely it is that the two abundances in question will in fact be observed to be different from one another. All scenarios were simulated for 500 iterations.

As an illustration of the use of scenario 1 within the *FishSampling* application, we might consider a farm in British Columbia, Canada, where the regulations specify that at least three pens per farm should be assessed with 20 fish per pen being sampled (Fisheries and Oceans Canada 2014). On this basis we suppose that a total of 60 fish were sampled from 3, 5, and 10 pens, on a farm consisting of 10 pens (Table 1 and Figure 2). The true abundance was assumed to be 3.0, while the lower and upper target limits were set to 2.7 and 3.3, respectively. The ICC was assumed to be 0.26. When only three pens were sampled, it can be seen that the likelihood of estimating an abundance between the two target limits was 0.25, or a probability of 25%. This rises to around 0.33 if five pens are sampled, and to 0.56 when all 10 pens are sampled. Here, it was found that the difference of “many pens” is obvious due to the substantial clustering effect. However, even when the ICC was lowered to 0.08, the benefit of “many pens” was still reflected in the likelihood values of 0.47, 0.59, and 0.77.

As an illustration of the use of scenario 2 within the *FishSampling* application, we also consider a farm in British Columbia, Canada, where there is a regulatory threshold of three mobile sea lice during the period when wild juvenile salmon are out-migrating (Fisheries and Oceans Canada 2014). On this basis, we consider a farm with 10 pens and use simulations from scenario 2 based on a total sample size of 60 (Table 1 and Figure 3). We set the (presumed) true abundance to be 3.3 and assume that there is a fair degree of clustering ($ICC=0.26$). The simulation allows the user to explore the probability that the estimated abundance will apparently be higher than the 3.0 threshold and compares this outcome to cases where the overall sampled total ($N=60$) is taken from 3, 5, or 10 pens on the farm. Using three pens, it can be seen that the likelihood of getting an estimated abundance over the 3.0 limit is only 0.60 (despite the fact that we “know” the true value to be 3.3). This rises to around 0.68 if five pens are sampled from, and to 0.76 when all 10 pens are sampled. While this level of clustering is not unrealistic based

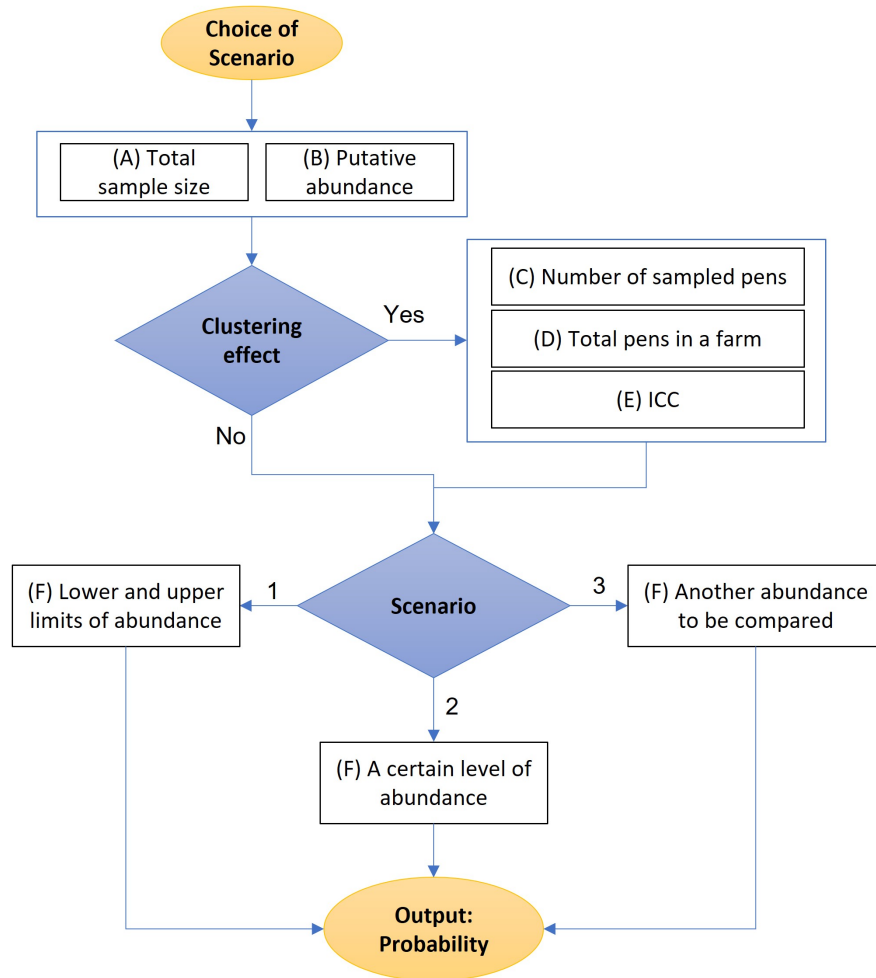


FIGURE 1 Diagram of the structure used in *FishSampling*. Any case requires total sample size (A) and putative abundance (B). If the clustering effect is chosen, the user should specify the number of pens to be sampled (C), the total number of pens on a farm (D), and the ICC (intraclass correlation coefficient) value (E). Next, the user decides which of the three scenarios they wish to explore, and the necessary input for each scenario (F) must be entered. Then, the simulation generates outputs relating to the likelihood that the purpose for which the salmon sampling is being carried out will be satisfied.

TABLE 1 Summary of simulated outcome likelihood values.^a For scenarios 1 and 2, two distinct ICC (intraclass correlation) values were applied, while scenario 3 utilized two different total sample sizes.

Number of sampled pens	Number of sampled fish per pen for scenarios 1 and 2	Scenario 1		Scenario 2		Scenario 3	
		ICC = 0.26	ICC = 0.08	ICC = 0.26	ICC = 0.08	Total sample size = 60	Total sample size = 120
10	6	0.56	0.77	0.76	0.81	0.90	0.97
5	12	0.33	0.59	0.68	0.71	0.84	0.86
3	20	0.25	0.47	0.60	0.68	0.78	0.79

^aDue to the nature of the randomness simulated in the application, any specific execution of the scenarios above will likely result in slightly different likelihood values. These were generated using the parameters specified in the text, based on 500 iterations (a value that can be modified by the user).

on empirical data (Revie et al. 2005, 2007), the “many pens” difference is most clear where significant clustering is present. Reducing the ICC to a value of 0.08 resulted in increased likelihood values of 0.68, 0.71, and 0.81 that the estimated abundance would appear to be over the 3.0 threshold for the cases of 3, 5, and 10 pens, respectively.

As an illustration of the use of scenario 3 within the *FishSampling* application, we consider a similar setting to that used in scenarios 1 and 2, but now propose a post-intervention comparison. Once again, a total of 60 fish were sampled from 3, 5, and 10 pens from a farm consisting of 10 pens (Table 1; Figure 4). The initial level of abundance

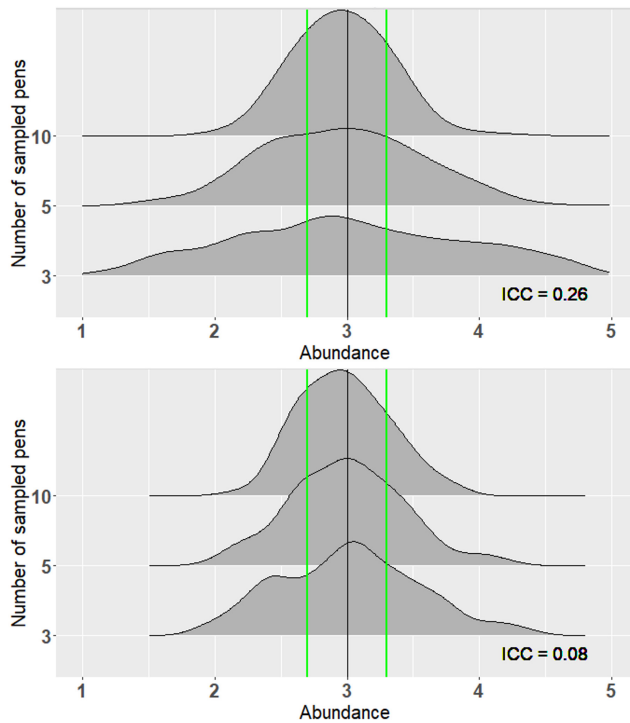


FIGURE 2 Distribution of abundance values from simulated output for scenario 1, which highlights the probability of achieving the desired level of precision in lice abundance estimation assuming certain inputs. The upper panel and the lower panel represent the results for ICC (intraclass correlations) values of 0.26 and 0.08, respectively. The likelihood of estimating the true abundance is represented by the area between the two green lines. The black and green lines represent true abundance and lower and upper limits, respectively.

was assumed to be 1.7 sea lice, with an ICC of 0.2 to represent a fair degree of clustering among pens. We then assumed that we wished to detect an approximate decrease of 25%, to an abundance of 1.3, and also assumed that the ICC would decrease to 0.09 due to a more homogeneous situation postintervention. Using three pens, the likelihood of detecting this level of reduction in abundance is 0.78. This increases to around 0.84 if five pens are sampled from, and to 0.90 when all 10 pens are sampled. It was still found that the “many pens” approaches brought benefits, although these were less marked due to the reduction in clustering, particularly subsequent to the intervention. In this case, doubling the total number of fish sampled (to 120) resulted in only minor changes, with likelihoods of 0.79, 0.86, and 0.97 that a decrease of 25% would be detected from the initial level of abundance, for the cases of 3, 5, and 10 pens, respectively.

DISCUSSION

Cost-effective interventions to manage marine ectoparasites rely on efficient monitoring systems (Myksvoll

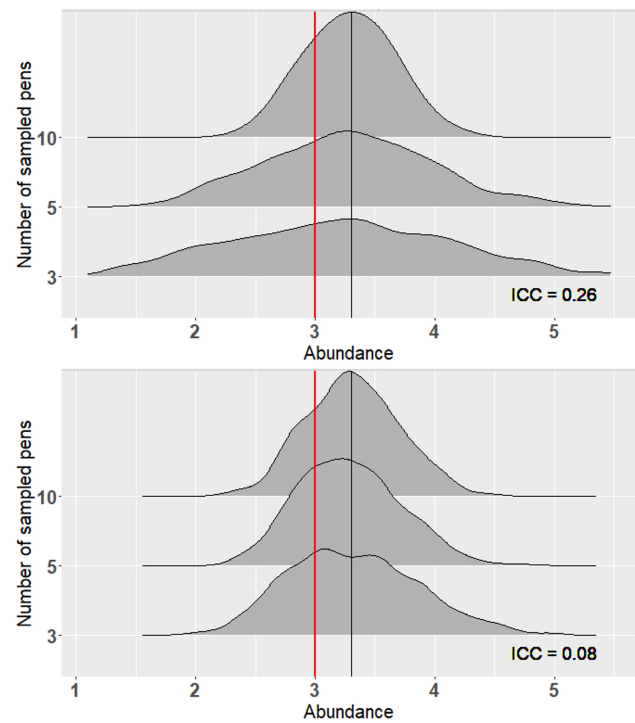


FIGURE 3 Distribution of abundance values from simulated output for scenario 2, showcasing the probability of correctly assessing lice abundance of a farm compared to a certain level of lice abundance with the assumed inputs. The upper panel and the lower panel represent the results for ICC (intraclass correlations) values of 0.26 and 0.08, respectively. The area to the right of the red line indicates this probability. The black and red lines represent the true and desired levels of abundance, respectively.

et al. 2018). Considering the substantial impact that sea lice infestation can have on the productivity of farmed salmon and conservation of wild fish, salmon-producing countries have developed regulations and guidelines indicating the number of fish and the number of pens that should be sampled as well as the frequency of sampling (Misund 2019). However, it is important to understand the level of precision around lice abundance estimation that can be expected when using a particular sampling strategy. Such an understanding should help prevent an overinterpretation of the precision of sample-based estimates, and ensure a more appropriate use of these results in the effective management of sea lice.

When a sample size needs to be determined in applied veterinary epidemiology, it is not uncommon for the situation to be inconsistent with a standard study design. Individual fish are aggregated into pens, and as such, simple formulae, which assume independence among fish, cannot be meaningfully applied to the calculation of sample sizes (Stevenson 2021). In such cases, “the advantage of simulation methods is that appropriate sample sizes can be estimated for complex study designs for which formula-based methods are not available” (Stevenson 2021). To develop this simulation-based application, we employed

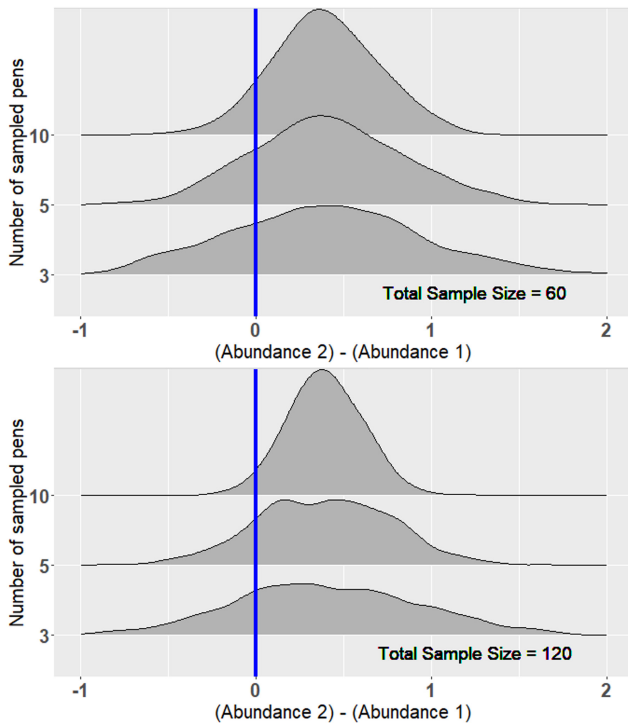


FIGURE 4 Distribution of the simulated differences in values between Abundance2 (higher abundance) and Abundance1 (lower abundance) for scenario 3, on a farm given the assumed inputs. The upper panel and the lower panel represent the results from total sample sizes of 60 and 120, respectively. The area to the right of the blue line highlights this probability. The vertical blue lines indicate simulated outcomes where the two abundances were estimated to be equal, so the area to the right of these blue lines represents the likelihood that a difference will in fact be observed.

basic programming and computer simulation to display the reliability and confidence limits of particular sampling strategies.

The modeling approach presented here allows stakeholders to adopt efficient sampling strategies for sea lice abundance estimation for a range of purposes. In particular, this application is useful in providing advice on the number of pens and the number of sampled fish per pen, under an assumption that the total number of sampled fish is a fixed limit. Previous studies have emphasized that in the presence of significant clustering, fewer pens and a greater number of fish will generate more precise estimation than using a larger number of fish from fewer pens (Revie et al. 2005, 2007; Jeong and Revie 2020). This application provides practical advice on how to decide on the number of fish and pens to sample, under varying levels of assumed clustering. There may of course be various sources of sampling bias, such as an increased likelihood of capturing poor performers with dip nets, which the approaches outlined here are unable to address (Jeong et al. 2021).

One limitation of this approach is that the modeled outputs are heavily influenced by the users' assumptions

of putative abundance and the level of clustering present (captured by the specified ICC value). This can appear to be something of a “chicken and egg” situation, as the purpose of sampling is to obtain an estimate of the sea lice abundance. The solution is to start with reasonable guesses around key assumptions by referring to previous sampling results. The model also allows for various “what-if” analyses. For example, what would be the implications on abundance precision if the actual ICC value was, say, 0.4 as opposed to the initial assumed value of 0.15? Another way to improve sampling precision is to enhance the counting skills of farm personnel, which should improve the estimation of sea lice abundance (Elmoslemany et al. 2013), as it has been documented that counters' competence can significantly affect estimates (Heuch et al. 2011). In addition, insufficient sample size can often be compensated for by engaging in more frequent sampling. Such approaches more effectively support the adoption of Bayesian estimation, where the use of informed priors can result in more precise estimates than would be the case under a frequentist model where noninformed priors are assumed (Musella et al. 2014). An exploration of the effects of sampling frequency on estimated precision is beyond the scope of this study, but it would be interesting for further studies to include both sample size and frequency in a more complete assessment of sea lice sampling designs. In addition, the development of novel technologies in the future may help mitigate concerns around sampling inaccuracy. For example, underwater camera systems and a machine-learning approach are being applied to count sea lice on salmon (Solvang and Hagemann 2018), and environmental DNA metabarcoding has been suggested as an accurate and cost-effective tool for monitoring marine parasite populations (Peters et al. 2018).

In summary, this study illustrated a novel approach to enhancing decisions around sample size determination for adequate sea lice infestation estimation on salmon farms. This article shows how to use a simulation-based approach to estimate sample size, when a formula-based approach has limited validity. Furthermore, the approach could be extended to other sampling contexts, particularly those relating to the estimation of ectoparasites where overdispersion is typically observed. This freely available application also contributes to an enhanced understanding of the main factors that affect the estimation of sea lice abundance at the farm level, ultimately supporting better management decisions to manage and control the impact of sea lice infestation on salmon farms.

ACKNOWLEDGMENTS

This work was partially funded through an FHF – Norwegian Seafood Research Fund (FHF 901411

– *Developing standardized methods for counting sea lice and calculating the amounts of sea lice*) and by the Ocean Frontier Institute's *New Models of Salmon Health Management* (OFI Module L) programme.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The R code used in this study is available at <https://github.com/jaewoonjeong/FishSampling>.

ETHICS STATEMENT

There were no ethical guidelines applicable to this study.

ORCID

Jaewoon Jeong  <https://orcid.org/0000-0002-1889-5346>

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