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# Monitoring parasitic abundance in cage-based aquaculture: the effects of clustering 

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#### Abstract

Most discussions of sampling protocols within the literature on monitoring aquatic parasites are based on the assumptions of simple random sampling. There has been a growing recognition within the fields of human and terrestrial veterinary epidemiology that data are often collected from individuals within clusters where such assumptions are not valid. These circumstances arise when monitoring ectoparasitic sea lice on Scottish salmon farms. In previous work the authors have demonstrated that significant intraclass correlation coefficients (ICC) values are associated with cage-level abundance of sea lice, particularly when the parasite reaches its adult stage of development. In this paper two sets of data from Scottish farms with ICC values for adult $L$. salmonis of 0.35 [0.08-0.72, $95 \% \mathrm{CI}]$ and for adult C. elongatus of 0.42 [0.14-0.66, $95 \% \mathrm{CI}]$ are used to investigate the implications of clustering. A Monte Carlo simulation approach is used to illustrate the effect of various sampling approaches. The protocols simulated reflect those typically used across a range of countries and production environments in which salmon are currently reared. By illustrating clearly from empirical data sets what is known by theoretical argument it is hoped that guidelines for sampling parasites, and disease monitoring more generally, within aquaculture will in future incorporate appropriate consideration of issues related to the clustering that is typically present in cage-based production systems.


## Introduction

As with most intensive farming systems, the viability of cage-based aquaculture is continually threatened by disease. The control and management of disease rely on the routine collection and interpretation of data associated with productivity, health and the environment. Such monitoring may be necessary as a regulatory requirement but more often it is undertaken in the interests of good practice to ensure populations remain healthy. Monitoring is often logistically difficult and costly and it is only useful if it can produce meaningful and reliable information. This requires the specification of sampling strategies for monitoring to be fit for purpose and in particular to ensure that neither too few nor too many data are collected.

## Sampling procedures for monitoring

There is a variety of regulations for reporting lice burdens on commercial farms but no international or standardised protocol for conducting lice counts exists (Treasurer and Pope, 2000). There is consequently variation in procedure among countries as seen in Table 1.

Table 1 Examples of current sampling protocols for monitoring sea lice infection levels on fish farms, illustrating typical numbers of fish and cages examined

| Country | No. fish | No. cages | Frequency |
| :---: | :---: | :---: | :---: |
| Ireland | 30 | 2 | biweekly |
| Norway | 20 | 2-3 | biweekly |
| Scotland | 5-10 | 4-6 | weekly |

Like many aquaculture monitoring systems the sampling procedures have evolved out of what is practical and expedient, and often without rigorous consideration of the sampling issues and the spatial configuration imposed by management practices (Revie et al., 2003). However, monitoring for disease raises important questions on how best to collect a sample of fish and how the sampling effort should take account of fish being clustered in cages.

## Results

## Empirical evidence of clustering for sea lice infestations

In order to investigate the effects of fish being clustered in cages, intraclass correlation coefficients have been empirically estimated using a range of chalimus and mobile L. salmonis and C. elongatus sea lice data sets collected at various sites (Revie et al., 2005). Table 2 shows the estimated intraclass correlations for a range of sites on the west coast of Scotland, together with their $95 \%$ confidence intervals. In all cases the intraclass correlation was found to be statistically significant ( $p<0.05$ ) irrespective of species or stage.

Table 2 Examples of spatial and temporal variation in intraclass correlation coefficients for sea lice infections associated with different species and stages

| Site | A | B | C | D | A | D |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| Date | Feb-02 | Aug-03 | Sep-03 | Aug-94 | Feb-02 | Aug-94 |
| Species | L. salmonis | C. elongatus | L. salmonis | C. elongatus | L. salmonis | C. elongatus |
| Stage | Chalimus | Mobile | chalimus | chalimus | mobile | mobile |
| Fish | 250 | 100 | 100 | 88 | 250 | 88 |
| Pens | 5 | 5 | 5 | 10 | 5 | 10 |
| ICC | $\mathbf{0 . 0 6}$ | $\mathbf{0 . 0 8}$ | $\mathbf{0 . 1 3}$ | $\mathbf{0 . 3 0}$ | $\mathbf{0 . 3 5}$ | $\mathbf{0 . 4 2}$ |
| Lower 95\% CI | -0.01 | -0.02 | -0.01 | 0.05 | 0.08 | 0.14 |
| Upper 95\% CI | 0.34 | 0.41 | 0.50 | 0.61 | 0.73 | 0.66 |
| Abundance | 13.8 | 2.3 | 1.9 | 5.4 | 10.1 | 6.4 |

## Sampling strategy illustrated by Monte Carlo simulation

In order to illustrate the effects of the ICC and various cluster sampling strategies on the accuracy of a sample in providing true estimates of population abundance, various sampling strategies were investigated using Monte Carlo simulation methods. The two data sets with the highest ICCs were chosen. In the case of $L$. salmonis mobiles at site A (ICC $=0.35$ ) the effect of the sampling strategy of taking 20 fish from each of 2 cages was contrasted with the sampling strategy of taking 8 fish from each of 5 cages. In the case of $C$. elongatus mobiles (ICC $=0.42$ ) at site $D$ the effect of taking 6 fish from each of 3 cages was contrasted with the sampling strategy of taking 2 fish from each of 9 cages.

Sampling simulations were executed 1,000 times and the mean of each sample obtained and compared to the true mean of the data set. The degree to which the sample means depart from the true mean is clearly seen from the plots of the distribution of the deviations for each sampling strategy shown in Figure 1. The strategies use the same total number of sampled fish, but when too few cages are sampled there is a high chance of a considerable deviation of the sample mean from the true mean. The graph illustrates that for site A the sampling strategy of 20 fish from 2 cages is highly likely to substantially over or under estimate the true mean. Similar differences are apparent for site D where more cages with fewer fish is again seen to be the superior strategy.


Figure 1 Results from Monte Carlo simulations illustrating the difference in sampling strategies on likely deviations form the true mean in the presence of ICC

## Discussion

It has been shown that the presence of clustering within cages has a significant effect on the ability of varying sampling strategies to accurately estimate the abundance of parasitic infection on fish farms. The key implications are straight-forward and well-understood by epidemiologists even if not currently applied in most aquatic monitoring programmes (McDermott and Schukken, 1994; Donner and Klar, 2000). Where significant clustering occurs (as has been demonstrated for sea lice on salmon farms) the best policy is always to sample the maximum number of cages possible even if this limits the number of fish per cage. This is in direct contrast to the policies currently adopted in Norway and Ireland where typically only two cages are sampled, with 20-30 fish from each. In the presence of clustering this is an inadequate sampling strategy to estimate farm-level abundance and also represents an inefficient use of sampling effort.

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