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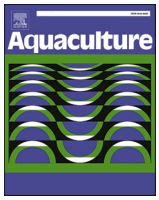

Abundance of sea lice larvae in plankton samples: determination of optimal sample sizes

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ABSTRACT

Sea lice infestations have been a major problem for the global salmon farming industry for several decades. To date, few studies have addressed the measurement of lice abundance in plankton samples and a standardized method to quantify sea lice larvae in water samples is still lacking. This study aims to: (1) evaluate the methods used to detect sea lice larvae based on published data and (2) to determine experimentally the volume of filtered sea water needed to obtain precise estimates of sea lice larvae abundance at different lice densities. Twenty-eight publications were reviewed with particular attention to sampling method and depth, total filtrated volume, analysed volume and nauplii and maximum copepodite densities. Moreover, plankton samples were obtained in and around salmon farms to evaluate the optimal water volumes required to estimate sea lice larvae abundance. This study provides a sampling and analysis strategy for quantifying larval sea lice in plankton samples from a cost/benefit point of view. Quantification of sea lice larvae in the plankton communities would be more precise than indirect methods used today (i.e adult sea lice attached on salmonids), and suitable for validation of modelling tools predicting the spatiotemporal dispersal of lice and, hence, the risk of infestation of salmon farms.

1. Introduction

The term 'sea lice' refers, sensu *lato*, to a group of ectoparasitic copepods from the family Caligidae that infect wild and farmed fish. In the North Atlantic, the salmonid specialist, *Lepeophtheirus salmonis* (Krøyer, 1837), and the teleost generalist, *Caligus elongatus* (von Nordmann 1832), are the most important and common sea lice affecting farmed Atlantic salmon, *Salmo salar* L. (Penston et al., 2004). The life cycle of these parasites comprises up to ten stages, of which three are free-swimming planktonic larval stages: nauplius I, nauplius II and the infective copepodite (Costello, 2006). The duration of each larval stage depends largely on temperature, with faster development of nauplius and copepodites at warmer sea temperatures (Pike et al., 1993; Costello, 2006). As part of the zooplankton community in the ocean, sea lice larvae drift with the currents and are exposed to local hydrographic conditions. Moreover, diel vertical migrations, thermoclines and salinity gradients (Heuch et al., 1995; Bricknell et al., 2006; á Norði et al., 2015; Crosbie et al., 2020) may generate changes in vertical and horizontal distribution of sea lice larvae. All these factors can determine the transmission of lice between hosts and other farmed or wild fish.

Sea lice infestations have been a major problem for the global salmon farming industry for several decades. In Norway, sea lice infestations on farmed salmon represent the most significant fish welfare issue for the salmon farming industry, with a total economic cost associated with preventing and reducing infestations estimated to exceed 370 million euros annually (Liu and Bjelland, 2014). Additionally, sea lice originating from farms may also infect wild salmonids with substantial negative effects on wild populations (Vollset et al., 2017). To predict the spatiotemporal spread of sea lice from salmon farms, and thereby foresee the need for treatment in the farms as well as the risk of infestation of wild salmonids, several complex modelling tools estimating the dispersal of sea lice larvae have been developed (Kristoffersen et al., 2018; Myksvoll et al., 2018). Validation of such models is based on various indirect ways of collecting empirical data on sea lice occurrence, including measurements of the numbers of sea lice being attached on wild salmonids as well as in sentinel cages stocked with hatchery salmon

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smolts (Sandvik et al., 2016; Salama et al., 2017; Myksvoll et al., 2018).

Quantification of larval lice abundance in plankton samples may be a more precise method for validation of modelling tools than the currently used methods, since such data would represent an estimate of lice occurrence at specific geographic positions in defined periods. However, few studies have addressed the measurement of sea lice abundance in plankton samples (e.g. Costelloe et al., 1996; Penston et al., 2008a, 2008b; á Norði et al., 2016; Skarðhamar et al., 2019). Several methods applying different filter mesh size, filtrated volume or sampled depth have been used in these studies, evidencing a lack of standardized method to quantify sea lice larvae in water samples. Different sampling methods may differ with respect to capturing spatiotemporal variability in occurrence of lice larvae, which may avoid direct comparison of sea lice larvae densities between different studies due to methodical differences (Jevne et al., 2021). In this context, filtrated water volume is important due to the low density of lice larvae in open waters, and the sample volume processed in the laboratory should be sufficiently large to obtain reliable and representative estimates of sea lice abundance.

The overall objective of the present study was to suggest a more standardized method for quantifying abundance of sea lice larvae in plankton samples. Specifically, our aims were to: (1) evaluate the methods used to detect sea lice larvae based on published data and (2) to determine experimentally the volume of filtered sea water needed to obtain precise estimates of sea lice larvae abundance at different lice densities.

2. Material and methods

2.1. Literature analysis

A comparison of the different sampling strategies used to estimate lice larvae from natural environments was conducted based on published data on *L. salmonis* and *Caligus* spp. The terms: ('sea lice' or '*Lepeophtheirus salmonis*' or '*Caligus elongatus*') and ('larvae', 'nauplii', 'copepodite') were used in Web of Science and Google Scholar in January 2021 to find all available literature related to sampling methods of sea lice larvae. Additional studies were included by reviewing the reference lists of studies returned by the initial search. All results were screened by material and methods to identify studies that were sampled with plankton collection devices. Twenty-nine publications were reviewed (24 peer-reviewed articles, 1 PhD thesis, 2 Master thesis and 2 conference proceedings publications), with particular attention to sampling method, depth, total filtrated volume, analysed volume and maximum nauplii and copepodite densities.

2.2. Experimental study: sample collection

To determine the volume of filtered sea water needed to obtain precise estimates of sea lice larvae, plankton samples were obtained between 3 and 25 August 2017 from 45 locations in and around three salmon farms in the Frøya municipality, Trøndelag county, central Norway (63°43′44″ - 08°51′04″). A gasoline trash pump (Honda WTX 20×) with a maximum discharge capacity of 450 l/min, attached to a weighted inlet hose and an outlet hose, both of 50 mm internal diameter, was used to sample plankton from 1, 3 and 9 m depth around aquaculture facilities. The sampled water volume was calculated by a digital flow meter (TM series water meters, Great plains Industries, Inc., Wichita, KS, USA) before the water was filtered through a 150 μm plankton net with a mouth diameter of 100 cm. A prefilter of 4 mm mesh was used to avoid large animals or macroalgae inside plankton samples. At each sampling point, 10 m^3 sea water was filtered. Then the plankton net was cleaned with seawater and the filtrate stored using 70% ethanol resulting a final volume of 500 ml.

The entire sample content was analysed using a stereomicroscope, by removing 5 m^l aliquots (subsamples) with a stempel pipette, after thorough mixing. A 5-ml subsample then represents a plankton sample from 0.1 m^3 water. All free-swimming planktonic sea lice lifecycle stages (i.e. nauplius I, nauplius II, copepodite) in each subsample were identified according to Schram (2004). Sea lice identification to species level was not possible due to similarities in L. *salmonis* and *C. elongatus* larval stages and the loss of colour patterns after preservation of the specimens in ethanol.

2.3. Statistical analysis

To evaluate the optimal water volumes required to estimate sea lice nauplii and copepodite abundance, we classified the lice density in each sample from very low (0–1 ind./m^3) to very high (up to 15 ind./m^3). Then, 22 samples were selected based on an initial screening that involved analysis of 20% of all the samples.

If the assumption of ideal mixing of the total sample and random subsampling is correct, each of the 5 ml aliquots can be assumed to be an independent observation of a Poisson distributed variable with expected lice density per 5 ml aliquot equal to the total number of lice counted in the entire sample divided by the number of aliquots. Under this assumption, it would be possible to perform calculations as if subsampling from a sample with any level of lice density is a Poisson process and evaluate the manual counting effort (number of aliquots) required for sufficiently precise estimation of sea lice densities. The standard error for the density estimate depends on both the number of aliquots and density, since the variance in the Poisson distribution equals the expectation. When analysing this type of data a common feature can be data overdispersion, i.e. that the variance is larger than expected from a Poisson process, due to e.g. clumping of lice in the sample (non-ideal mixing). If overdispersion is found, the negative binomial distribution should be used in further analyses. The hypothesis that the observations follow a Poisson distribution was tested for each sample by calculation of the Chi-square statistic and determination of the *p*-value from the appropriate Chi-squared distribution. The assumption that the observations were identically and independently distributed was tested further by analysing if sequence order affected expected density. Finally, we illustrated how the precision of the estimates can be evaluated as a function of counting effort and density. The (1-α)% confidence interval for the mean of a Poisson distribution is:

$$\frac{1}{2}\frac{\chi^2(\alpha/2;2k)}{n} \le \lambda \le \frac{1}{2}\frac{\chi^2(1-\alpha/2;2k+2)}{n}$$

where k is the (expected) observation from accumulated sample number n, and each aliquot is assumed to have the same constant rate λ and $\chi^2(gdf)$ is the quantile from the chi-square distribution with df degrees of freedom.

3. Results

The comparison of sampling strategies used to estimate sea lice larvae from natural environments is shown in Table 1 (at the end of the manuscript). Most of the studies focused on the species L. *salmonis*, and secondly, on *C. elongatus*. Horizontal tows were the most frequently used sampling method, but vertical hauls and pumps were also commonly used to sample sea lice larvae. Thus, sampling was mostly carried out close to the surface (0–0.5 m) using a mesh size of 150 μm. A major issue with the analysis of sampling strategies was the lack of information regarding water volume sampled and sample volume analysed in most of the papers considered in this study.

Maximum lice densities reported in the literature are shown in Table 2. Maximum densities of planktonic lice were usually reported in samples collected close to the surface with horizontal tows. The highest copepodite density was 423 ind./m^3, reported in a coastline location. The highest densities for copepodites were consistently reported from shoreline areas like estuaries or river mouths. The highest nauplii densities were found in fish farming areas with a maximum of 66 nauplii/m^3

Table 1
Literature review of sampling methods.

	More cited → Less cited							
Lice species	*L. salmonis* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27	*C. elongatus* 1, 7, 8, 16, 18, 19, 21, 22, 26, 27.		*C. clemensi* 24, 25		Others (*C. rogercresseyi*[15], *L. pollachius*[26])		
Sampling method	Horizontal tows 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 23,, 27	Vertical hauls 8, 20, 21, 22, 24, 25, 26, 27, 28, 29.		Pump 1, 4, 19, 20, 27		Others (Light lure- pump[1], Go-flo bottles[27])		
Mesh (microns)	150 2, 3, 4, 8, 9, 10, 11, 13, 15, 17, 18, 19, 20, 21, 23, 24, 25, 26, 28, 29.	140 5, 6, 8, 28.		200 8, 22, 28.		Others (60[1], 68[21], 90[27], 95[7], 180[27], 250[12])		
Volume sampled	Unspecified 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 14, 15, 16, 17, 18, 25, 26	> 10 m^3 9, 13, 21, 23, 27	1 – 3 m^3 20, 22, 24, 27, 28, 29.	< 1m^3 1, 20, 27	3.1 – 6 m^3 19, 21, 24	6.1 – 10 m^3 27		
Volume analysed	Unspecified 2, 3, 4, 5, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 21, 22, 23, 24, 25, 27, 28.	Entire 1, 18, 19, 20, 26, 28.		Aliquots (four 5 ml) 6, 7		50% 26, 28, 29.		
Sampling depth	Surface 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 18	Entire water column 7 m[22, 28, 29], 10m[27,28], 17 m[20], 18m[24,25], 40 m[21]	1m 7, 19, 20, 21, 27	5m 1, 7, 9, 23, 27	10 m 1, 15, 20, 27	Others (2m[12.23], 3m[20.27], 4m[19.27], 6m[12.19.20], 7m[26], 8m[12], 14m[20], 15m[1,15], 17m[20], 20m[1,23])		

1.Gravil, 1996; 2. Costelloe et al., 1996; 3. Costelloe et al., 1998a; 4. Costelloe et al., 1998b; 5. McKibben and Hay, 2002; 6. McKibben and Hay, 2004; 7. Penston et al., 2004; 8. McBeath et al., 2006; 9. Penston et al., 2008a, 2008b; 10. Penston et al., 2008b; 11. Penston and Davies, 2009; 12. Morton et al., 2010; 13. Penston et al., 2011; 14. Salama et al., 2011; 15. Molinet et al., 2011; 16. Adams et al., 2012; 17. Salama et al., 2013; 18. Á Norði et al., 2015; 19. Á Norði et al., 2016; 20. Nelson et al., 2018; 21. Harte et al., 2017; 22. Øvrelid; 23. Salama et al., 2017; 24. Byrne et al., 2018a, 2018b.; 25. Byrne et al., 2018b; 26. Dimmen, 2019; 27. Skarðhamar et al., 2019; 28. Jevne et al., 2020; 29. Jevne et al., 2021.

Table 2
Sample depth, larval stage and location of the maximum sea lice densities reported in published bibliography. Ls: *Lepeophtheirus salmonis:* Ce: *Caligus elongatus; Cr: Caligus rogercresseyi*; SSL: several species of sea lice. Sampling works carried out at surface water are represented as 0–0.5 m water depth. Capital letter in 'X' means that majority of the found sea lice were belong this larval stage.

	Sampling method	Sample depth	Sealice density (ind/m^3)		Larval stage		Location		
			Maximum	Mean	Nauplii	Copepodid	Coastline	Offshore	Farm
Gravil, 1996	Pump	5 m	220 (Ls)						x
			80 (Ce)						
Costelloe et al., 1996	Horizontal tow	0–0.5 m	66 (Ls)		**X**	x			x
Costelloe et al., 1998a	Horizontal tow	0–0.5 m	16 (Ls)				x		
Costelloe et al., 1998b	Horizontal tow	0–0.5 m	16 (Ls)				x		
McKibben and Hay, 2002	Horizontal tow	0–0.5 m	423 (Ls)			x	x		
McKibben and Hay, 2004	Horizontal tow	0–0.5 m	123 (Ls)			x	x		
Penston et al., 2004	Horizontal tow	0–0.5 m	543 (SSL)				x		
	Horizontal tow	0–0.5 m	11.2 (SSL)		x	x		x	
Penston et al., 2008a	Horizontal tow	5 m		5.6 (SSL)	x				x
	Horizontal tow	0–0.5 m		1.8 (SSL)		x			x
	Horizontal tow	5 m		0.3 (SSL)	x			x	
Molinet et al., 2011	Horizontal tow	0–0.5 m	1.03 (Cr)			x			
Á Norði et al., 2015	Horizontal tow	0–0.5 m	3.2 (Ls)		**X**	x			x
	Horizontal tow	0–0.5 m	0.96 (Ce)		x	**X**		x	
Á Norði et al., 2016	Horizontal tow	1 m	4.2 (Ls)		x				x
	Horizontal tow	1 m	1.1 (Ce)		x				x
Nelson et al., 2018	Pump	5 m		6.8 (Ls)	**X**	x			**X**
	Vertical haul	1-17 m		0.24 (Ls)	**x**	**X**		x	
Harte et al., 2017	Horizontal tow	1 m	14.2 (Ls)			x	x		
		?	1.1 (Ce)			x			
Øvrelid, 2017	Vertical haul	1-7 m		22.25 (SSL)	**X**	x			x
Salama et al., 2017	Horizontal tow	2 m	0.18 (Ls)		x				
Byrne et al., 2018a	Vertical haul	1-18 m		1.3 (SSL)	**X**	x			x
	Vertical haul	1-18 m		0.8 (SSL)	x			x	
Byrne et al., 2018b	Vertical haul	1-18 m	1.5 (SSL)						x
Dimmen, 2019	Vertical haul	1-7 m	6 (SSL)		**X**	x			x
Skarðhamar et al., 2019	Go-Flo bottles	3 m	30 (SSL)			x	x		
	Vertical haul	0–10 m	12.8 (SSL)			x		x	
	Horizontal tow	4 m	1 (SSL)			x	x		
	Pump	3 m	9 (SSL)			x		x	
Jevne et al., 2020	Vertical haul	1–10 m		4.78 (SSL)	**X**	x			x
Jevne et al., 2021	Vertical haul	1-7 m	12.3 (SSL)	10 (SSL)	**X**	x			x

VFG, PSJ and IU conceived the ideas and designed methodology; VFG, EMU, PSJ, KTG, PK and IU collected the samples; VFG, NCC, PSJ and KTG analysed samples at the laboratory; VFG conducted the literature analysis, OHD and IU performed statistical analysis; VFG and IU led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

reported by McKibben and Hay (2002) within a salmon cage.

Regarding to the experimental part of this study, the lice densities in the plankton samples analyses varied from 0 to 24 ind./m^3. In total 749 nauplii (min = 0, max = 163, mean = 34), 17 copepodites (min = 0, max = 3, mean = 0.77) and 3 adults (min = 0, max = 1, mean = 0.14) were found in the 22 samples. Hence, the numbers of copepodites and adults were too low to allow tests for distributional properties.

There was no evidence from the Chi-square tests suggesting that the nauplii data departs from a Poisson distribution, and all samples have *p*-values well above 0.5 (Table 3). Therefore, it is reasonable to assume that subsample counts are Poisson distributed with expected number of lice in each aliquot estimated as the mean count. The goodness of fit is illustrated in Fig. 1 for two samples, one with low and one with high densities.

Additionally, sampling order was analysed in order to check whether the lice subsampling can be considered identically or there is any effect on the expected number of lice in an aliquot, i.e. that all variation is not random but some can be explained by aliquot sequence order. For the nauplii samples, we found a significant negative trend in expected number of lice with subsampling order (Fig. 2; Poisson glm; $p < 0.001$), indicating that the probability of detecting lice is highest in the first aliquots.

Estimate precision can be evaluated analytically once the trend is removed and subsample lice abundances can be assumed to be independent and identically Poisson distributed. By doing this we can illustrate how large the counting effort for a given lice density must be to obtain an estimate with a given precision. We have illustrated two types of statistical uncertainties for different densities and counting efforts; first one for estimating the actual density at sea where our "entire sample" is considered as "just a sample", illustrated by the confidence intervals in Fig. 3 (red dashed lines). In this case, the 95% confidence interval, that illustrates the expected estimation uncertainty for the mean density from the "entire sample", will be fairly wide even after all 100 subsamples are counted.

The second type of statistical uncertainty originates from the subsampling procedure, i.e. when estimating the density of lice observed in the "entire sample" from a number of subsamples. These confidence intervals (blue dashed lines) were obtained by random sampling of subsamples from the original ones (10,000 resamples for each number of subsamples. For example, for $n = 10$ subsamples we randomly sampled 10 of the 100 subsamples and calculated the mean number of lice for these, and then repeated this procedure 10,000 times. We thereby obtained the sampling distribution for the mean for this specific sampling effort, from which we calculated the 2.5% and 97.5% limits that are required for determining the 95% confidence interval. These confidence intervals will inevitably shrink towards zero with increasing number of subsamples counted. The relevance of the two ways of estimating statistical uncertainty depends on the goal of the analyses.

The relative uncertainty, for each of the two ways of evaluation uncertainty, was illustrated by simulation from a range of realistic lice densities: 0.1, 0.5, 1, 5, 10 and 20 lice per m^3 water. The relative uncertainty was estimated as the point and confidence estimates divided by the density we simulated from. The horizontal black dotted lines are thereby all at 1, while the coloured (red and blue) dotted lines shows the relative deviation of the 95% confidence intervals from the real densities.

In general, the relative uncertainty decrease with increasing expected lice density and counting effort. The simulations indicate that precise estimation of very low, but realistic, lice densities in the sea on basis of plankton sampling (< 0.5 lice m^3) would require a very high counting effort (Fig. 3A). When the lice density is 0.5 lice per m^3 the relative uncertainty levels out when around 40–60% of the sample is analysed, but the relative uncertainty for estimation of lice densities in the sea is still high when the entire sample is analysed (Fig. 3B). When lice densities increase above 0.5 lice per m^3 the relative uncertainty levels out at gradually lower counting efforts, and analysis of 20–40% of the samples, (corresponding to a water volume of 2–4 m^3) would yield a relatively low expected uncertainty, both with respect to estimating the density of lice larvae in the sea and in the individual samples (Fig. 3C, D, E and F).

Table 3
Chi-square test results for all nauplii samples. The test is performed only for samples with 5 or more lice in the entire sample.

Sample ID	Min	Max	Mean	Var	Chi Sq	df	p-value
1	0	0	0,00	0,00	NA	NA	NA
2	0	0	0,00	0,00	NA	NA	NA
8	0	1	0,01	0,01	NA	NA	NA
56	0	0	0,00	0,00	NA	NA	NA
73	0	0	0,00	0,00	NA	NA	NA
119	0	1	0,01	0,01	NA	NA	NA
33	0	0	0,00	0,00	NA	NA	NA
47	0	1	0,03	0,03	NA	NA	NA
53	0	1	0,07	0,07	0,03	1,00	0,85
94	0	0	0,00	0,00	NA	NA	NA
99	0	2	0,10	0,11	0,79	2,00	0,68
107	0	1	0,08	0,07	0,05	1,00	0,82
30	0	0	0,00	0,00	NA	NA	NA
58	0	2	0,17	0,16	0,07	2,00	0,97
62	0	1	0,03	0,03	NA	NA	NA
68	0	2	0,22	0,21	0,01	2,00	1,00
106	0	2	0,17	0,18	0,63	2,00	0,73
97	0	5	1,63	1,47	0,63	5,00	0,99
102	0	5	1,09	1,13	3,09	5,00	0,69
103	0	4	0,87	1,02	2,68	4,00	0,61
105	0	5	1,38	1,13	2,87	5,00	0,72
114	0	5	1,63	1,53	2,60	5,00	0,76

4. Discussion

The literature analysis carried out in this study revealed that a standardized method for estimating lice larvae densities in plankton samples is not established, although most of the studies have sampled plankton from similar depths by using plankton nets with 150 μm mesh size. Main issues in previous studies were the lack of information about samples volumes and/or the processing method in the laboratory. Horizontal tows were the sampling method mostly used, although vertical hauls and pumps were also commonly used for plankton collection. Proper sampling method selection is essential to maximize accuracy, where variables such as speed and/or duration of tows, plankton net mouth opening and mesh size or water depth may affect the efficiency of the sampling (Jacobs and Grant, 1978). Moreover, if sampling was conducted close to salmon farms, plankton pumps appear to be a better alternative than plankton nets, because plankton nets may become entangled in ropes and nets in the fish farms (Nelson et al., 2018). Plankton pumps may also be less affected by wind and waves than plankton nets (Skarðhamar et al., 2019). However, the capacity of a plankton pump should be sufficiently high, and optimally no less than 200 l/min, to minimise the risk of copepodites avoiding the suction hose inlet (Jacobs and Grant, 1978).

Although lice larvae can be successfully sampled with several different plankton collection methods, the water volume filtered will inevitably influence the accuracy of the estimates. Samples from small water volumes, as for instance would be the case when using Go-Flo bottles, may result in high uncertainty (Harris et al., 2000; Skarðhamar et al., 2019) due to natural patchy distributions of lice in the water column. Thus, the water volume sampled should be sufficiently large to obtain reliable and representative estimates of lice abundance. Horizontal tows allow sampling from large water volumes, which may be calculated from net mouth area and towing speed or flowmeters. However, information on total sampling volumes are not provided in many of the previous studies that have attempted to estimate lice larval densities. Large volumes of water (i.e. 6–10 m^3) can also be sampled and filtered using pumps, as has been shown in this study and previous

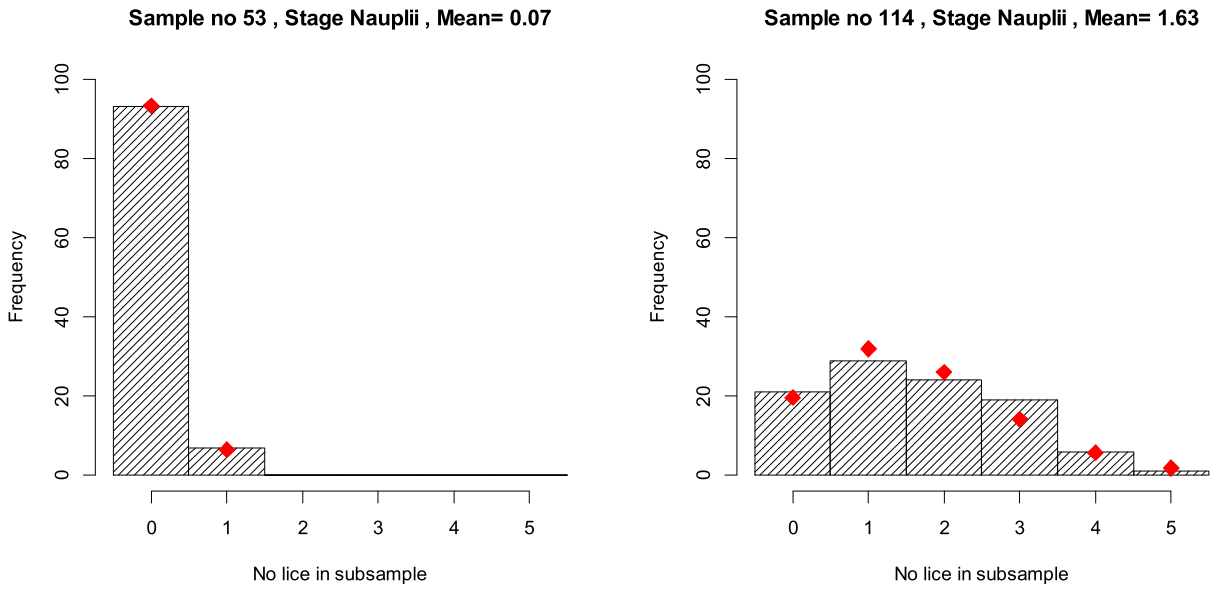


Fig. 1. Goodness of fit to the Poisson distribution for sample no. 53 and 114. Histogram bars show observed frequencies while red diamonds show theoretical expectations according to the appropriate Poisson distributions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

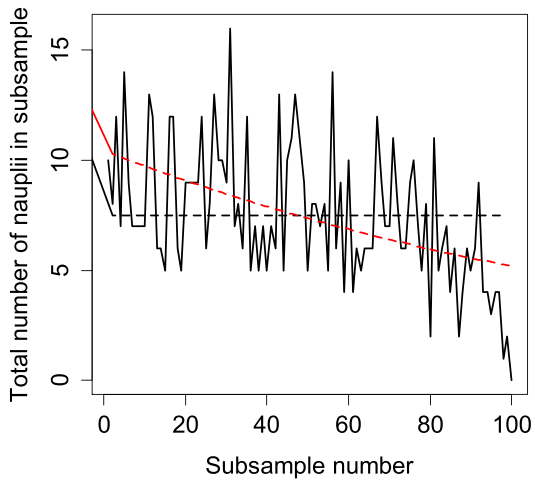


Fig. 2. Total number of nauplii per subsample number, i.e. we summed lice abundances for each subsample number over all 22 samples. Solid line shows the observations, dashed black line the mean number of lice per subsample and the dashed red line the fitted generalised linear model (glm) for Poisson distributed response. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

studies (Á Norði et al., 2016; Skarðhamar et al., 2019).

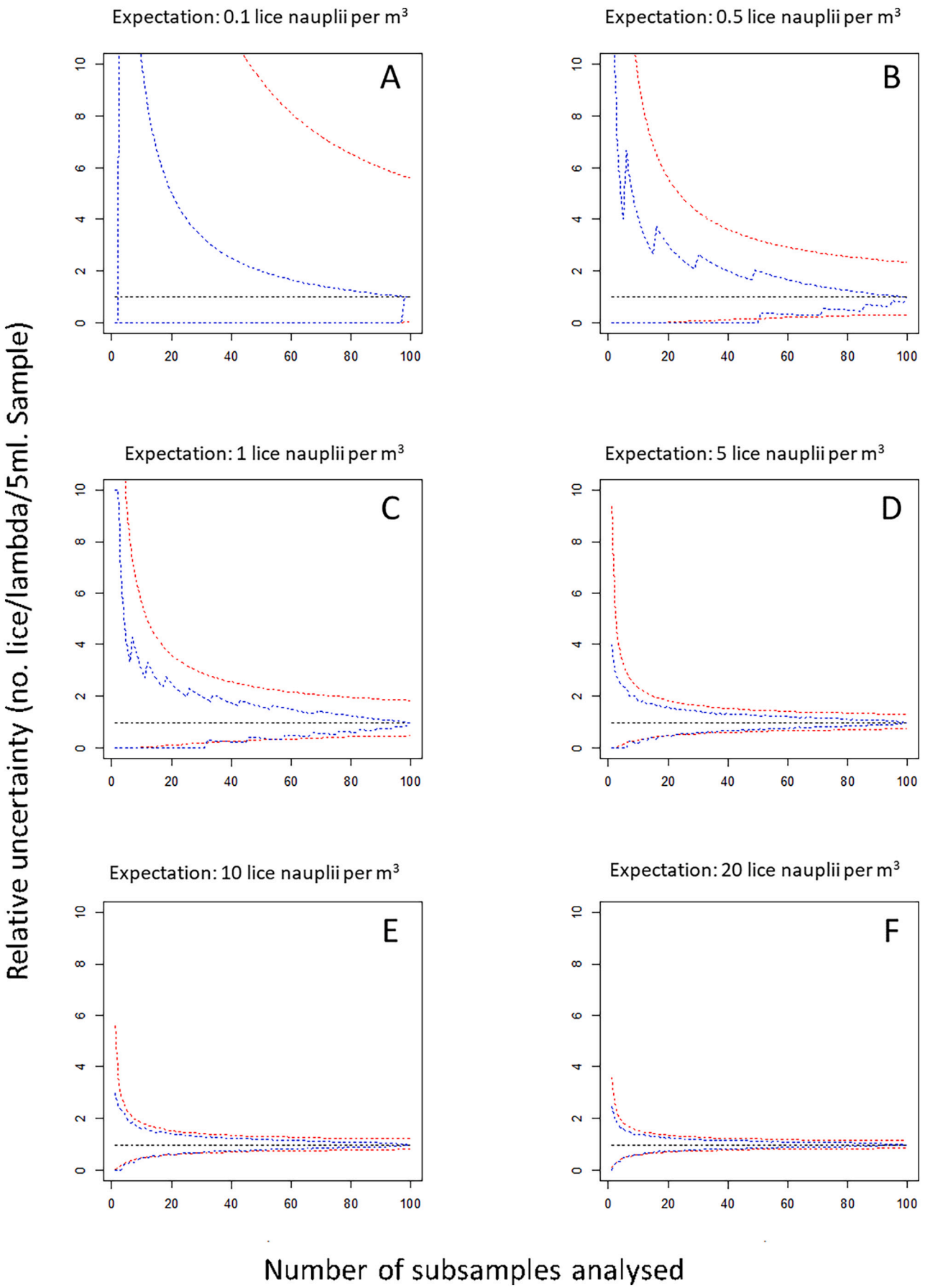
Different sampling methods may also differ with respect to capturing spatiotemporal variability in occurrence of lice larvae. The sampling in most of the previous studies were carried out close to the surface, and maximum densities reported in the bibliography were also mainly observed at this depth. However, information on vertical distribution of lice larvae may be interesting in order to develop tools and methods for preventing lice infestation in salmon farms (Coates et al., 2020). Better resolution at horizontal and vertical scales can be obtained using pumps, which offer the possibility of sampling specific points or water depths. This should be taken into account in studies aimed at assessing spatio-temporal variability in occurrence of lice larvae.

The most frequently used mesh size for collection of lice larvae was 150 μm, which appears to be adequate in relation to the minimum width of 165 μm (Schram, 2004) reported for lice nauplii I. Larger mesh size could be used if copepodites only were targeted, but the mesh size should still not be larger than 200 μm since this could lead to the loss of organisms.

One of the main knowledge gaps regarding optimal assessment of lice larvae densities is what sample volume will be sufficient for a reliable estimation of lice larvae density. In this context, sample volume would correlate with the effort needed for manual counting of lice larvae in the plankton samples. Since large volumes of water are necessary to collect representative lice densities in natural environments and the analysis of large plankton samples is very labour intensive, partial analysis of the plankton samples could be relevant if this approach yielded reliable estimates of lice density.

Our analysis show that the counting effort required to obtain precise estimates of lice abundance is related to the density of lice. According to literature analysis, lice densities in open-waters are generally very low (0.1 to 0.8 lice larvae per m^3; Byrne et al., 2018a, 2018b). However, these densities may be an order of magnitude higher around salmon farms (Nelson et al., 2018), and values of 66 nauplii/m^3 have been recorded within the sea cages (Costelloe et al., 1996). The presence of copepodites is assumed to increase far from salmon farms (Costelloe et al., 1996), and may appear in high numbers close to the shore line or river mouths (Costelloe et al., 1998a, 1998b; McKibben and Hay, 2002, 2004; Penston et al., 2004; Harte et al., 2017).

Lice densities in this study varied from 0 to 0.1 ind./m^3 to 24 ind./m^3, which is representative of previously reported lice densities in farming areas in Norway. Depending on lice density it would be possible to decide the adequate number of aliquots to analyse, where in general, the required counting effort increase with decreasing density. For lice densities above five lice per m^3 a partial analysis of 20% of the sample would be enough to provide as reliable estimates as possible. Lower densities, down to 1 lice per m^3, would require subsampling of at least 50% of the samples. However, it would be virtually impossible to analyse large enough samples to determine very low lice densities with high precision. It may therefore be sensible to adopt an analysis strategy that involve that lice densities below a specific limit should be quantified as a "density below that limit". According to the simulations carried out in this study it would be reasonable to suggest that this limit would be set to below one lice per m^3 water sampled, depending on the analysis effort that realistically can be devoted to manual counting of lice. This



(caption on next page)

Fig. 3. Relative uncertainty for 0.1 (A), 0.5 (B), 1 (C), 5 (D), 10 (E) and 20 (F) lice per m^3 water. The horizontal black dotted lines gives the relative uncertainty of the density scaled to 1, the blue dotted lines the relative estimation uncertainty of the density in the entire sample (95% confidence interval) originating from the subsampling procedure, while the red dotted lines illustrates the relative uncertainty of the density estimate (95% confidence interval) when we, in addition to the subsampling, also consider the entire sample as a sample from sea. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

may however change with development of new methods within molecular biology that could allow quantification of the occurrence of lice down to molecular level within plankton samples.

Our results indicated that the standard way of collection of aliquots for analysing plankton samples may result in a sampling bias, in terms of a higher probability of detecting lice in the first aliquots compared to the last aliquots. The cause of this bias is unknown, but it may be assumed that the physical properties of lice larva involve that they have a higher probability of being found in the first aliquots, even though the plankton sample is thoroughly mixed before extraction of an aliquot. This has also been observed in some organisms such as cladocerans, which may float in the surface of the sample (Harris et al., 2000). Thus, the addition of chemical products that promotes sinking all organisms to the bottom may be necessary in order to avoid this bias and a possible overestimation of the lice densities, when only parts of a plankton sample is analysed. Nevertheless, if the relationship between the probability of detecting lice and subsampling sequence is known it would be possible to account for this bias by developing appropriate statistical models.

5. Conclusions

Our results suggest that development of a standardized method to quantify sea lice larvae in water samples for monitoring programs and model validation is necessary. It would be important to collect plankton samples from sufficiently large water volumes to obtain reliable and representative estimates of lice abundance. If the plankton samples are collected from too small water volumes the accuracy of the estimates may be low. According to our results and published bibliography, this volume could be in the range 6–10 m^3 (á Norði et al., 2016; Skarðhamar et al., 2019). Furthermore, plankton pumps would be the preferred sampling method if the information about horizontal and vertical distribution is required and if the sampling occurs close to fish farms. Moreover, the capacity of the pump should be no less than 200 l/min to minimise copepod avoidance. The mesh size of the plankton net should not exceed 150 μm to ensure retention of all larval stages of sea lice. Furthermore, the volume of sample that needs to be analysed in the laboratory might also depend on the lice densities. Manual counting of lice larvae from large water volumes is a very time-consuming task and more efficient analysing methods are necessary. In this study, we have used lice numbers from plankton samples, to evaluate the optimal number of subsamples to estimate the abundance of salmon lice nauplii and copepodites in relation to variation in larvae density. Our results indicate that a partial analysis of 20% of the sample would be adequate to provide reliable estimates of lice densities at above five lice per m^3. An analysis of at least 50% of the sample is necessary to increase the accuracy in lice densities down to one lice per m^3. Densities below this could be best regarded just as '$<$ 1 lice per m^3'.

Authors' contributions

VFG, PSJ and IU conceived the ideas and designed methodology; VFG, EMU, PSJ, KTG, PK and IU collected the samples; VFG, NCC, PSJ and KTG analysed samples at the laboratory; VFG conducted the literature analysis, OHD and IU performed statistical analysis; VFG and IU led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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