A method of estimating *in situ* salmon louse nauplii production at fish farms

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**ABSTRACT:** Abundance and depth distribution of planktonic sea lice were investigated in relation to hydrodynamics and diurnal solar insolation at a salmon farm in Sundalagið, Faroe Islands. Plankton surveys were conducted by surface tows with a plankton net around the farm and by using a plankton pump at 1, 4 and 6 m depth in a fish cage. The entire sample content was investigated under a stereomicroscope and sea lice were identified. Sea lice of the species *Lepeophtheirus salmonis* and *Caligus elongatus* were present at the farm. Nauplii dominated the planktonic stages (>95%) while copepodids were absent from most samples. The highest observed copepodid density was 0.3 ind. m⁻³, which is within the range found in open water. No diurnal vertical distribution pattern was observed for salmon lice nauplii in the net cages, which were most abundant in the top meter of the water column, and density decreased with depth. At 1 m depth, nauplii density was inversely proportional to the current speed at the same depth. From this relation, and the abundance of adult female sea lice on the farmed fish, the *in situ* nauplii production was calculated to be within 26–68 nauplii female⁻¹ d⁻¹. The lower end of this range is similar to production rates suggested by laboratory studies at similar temperatures (7.8°C).

**KEY WORDS:** *Lepeophtheirus salmonis* · *Caligus elongatus* · Sea lice · Infectious copepodid · Nauplii · Sea cage farming · Aquaculture · Salmon louse

**INTRODUCTION**

The salmon louse *Lepeophtheirus salmonis* is a naturally occurring parasitic copepod in the northern hemisphere and has been a subject in the salmon farming industry since the onset of commercial salmon aquaculture (Pike & Wadsworth 1999). Typically, salmonids are farmed in floating net pens with free water exchange. This ensures removal of waste and supply of oxygenated water. However, the free water exchange also includes exchange of pathogens such as salmon lice, which transmit between hosts as planktonic larvae (Pert et al. 2014). In addition to *L. salmonis*, salmon farmed in the North Atlantic Ocean may also be infected by the teleost generalist *Caligus elongatus* (Nordmann, 1832). However, as *L. salmonis* is more pathogenic to farmed salmon, it has been studied more intensively than *C. elongatus* (Boxaspen 2006).

Both species have 3 planktonic stages; 2 nauplius stages prior to the infective copepodid. Both nauplii and copepodids are non-feeding and drift with the currents (Pike & Wadsworth 1999, Boxaspen 2006). However, they show behavioural traits that play a role in host-finding (Mordue & Birkett 2009), and they also respond to environmental stimuli; e.g. avoidance of freshwater (Bricknell et al. 2006), diel vertical migration (Heuch et al. 1995) and recently, the likelihood of nauplii vertically seeking the highest possible temperature has been discussed (Johnsen et al. 2014, á Norði et al. 2015).

Salmon farms are identified as contributors to the salmon lice infection pressure of both farmed and wild salmonids (Morton et al. 2011, Aldrin et al. 2013, Serra-Llinares et al. 2014, 2016). This is especially the case towards the end of the farming cycle, when the prevalence of adult female lice on the farmed fish can be high (Torrissen et al. 2013). There are numer-
mechanisms other than chemical agents. (Aaen et al. 2015) has called for sea lice control to chemotherapeutics in salmon lice populations. However, the development of resistance pathogen transmission between farmed and wild below sub-clinical levels, and in the mitigation of role in keeping the sea lice levels on farmed fish use of chemotherapeutics has played an essential tinuously since the onset of farming (FAO 2014). The production of farmed salmon has increased con-

Various technologies that aim to prevent the infectious copepodids from reaching the farmed fish e.g. shielding skirts, snorkel cages, underwater feeding and light, light traps, electric fences and mussel beds are under development or have been developed (Molloy et al. 2011, Frenzl et al. 2014, Lien et al. 2014, Aaen et al. 2015). The majority of these technologies are based on the copepodids being most abundant in the upper few meters of the water column (McKibben & Hay 2004, Penston et al. 2004, Molinet et al. 2011), and responding to light stimuli (Hevrøy et al. 2003, Genna et al. 2005). However, observations on the target organism around fish farms are quite limited, and basic information is still scarce.


In the vicinity of fish farms, L. salmonis nauplii are the most common developmental stage found, while observations of copepodids are scarce (Costelloe et al. 1996, Morton et al. 2011). The nauplii-to-copepodid ratio decreases with distance to the farms, due to a decrease in nauplii density (Costelloe et al. 1996). Highest copepodid densities are commonly observed in near-shore environments, ideal for intercepting migrating hosts (Costello 2006). This is most probably a result of the copepodids drifting with the surface currents influenced by wind (Salama & Rabe 2013, Asplin et al. 2014, á Norði et al. 2015).

In this paper, 2 snapshots of sea lice abundance at a fish farm are presented in relation to the concurrent hydrodynamics, solar insulation and sea lice counts on the farmed fish. Further, it is demonstrated how an in situ estimate on the nauplii production can be accomplished from such high-resolution measurements.

**MATERIALS AND METHODS**

**Study site**

The study was conducted at a fish farm in the northern part of Sundalagið, Faroe Islands, towards the end of the farming cycle when sea lice prevalence on farmed fish was at its maximum. The farm consisted of 18 net cages mounted below a floating platform in arrays of 3 x 6 (Fig. 1). The dimensions of the net cages were 24 x 24 x 20 m. No artificial light source was applied at the farm, and an automatic feed dispenser continuously fed the fish at the surface during daylight. Surface water temperature and salinity ranges in the area are quite small, ranging from 6 to 11°C and 32 to 35, respectively (Gaard et al. 2011, á Norði et al. 2015). No sharp pycnocline, but a gradual change in salinity and temperature with depth occurs in the upper 20 m due to wind-induced mixing (á Norði et al. 2015).

A spatial and a temporal survey of sea lice distribution were conducted. First, on February 7, 2014 the spatial distribution of sea lice was investigated at 1 m depth at 14 different sites inside and around the fish farm (Fig. 1). Samples were taken in daylight within 5 h at the time when the fish farm was at the end of the grow-out period and held 675 000 salmon.

The second survey investigated temporal changes in sea lice abundance in one of the net cages at the farm. Plankton samples were consecutively taken at 1, 4 and 6 m depth every third hour over a 24 h period. The survey began on May 6, 2014, at which time harvesting had commenced with net cage and adjacent cages being in full production. The investigated net cage held 35 000 salmon. During the measurements, the solar insulation was recorded hourly with a digital Quantum Scalar Laboratory sensor (QSL – 2100, Biopsherical Instruments).

During both surveys, temperature, salinity and water current measurements were performed adjacent to the cages for 24 h at 1 m depth with a Sea-guard RCM SW from Aanderaa (www.aanderaa.com). In order to collect information on the water current field around the farm, the deployments were conducted on diagonal corners at the farm. The net cages were virtually without biofouling, as observed by an underwater camera.
In situ nauplii production

Inside the cages and outside the walkway of the floating platform, plankton was sampled by a plankton pump model 23.570 (KC-Denmark) with a mesh size of 150 µm and a capacity of 300 l min\(^{-1}\), from 0 to 40 m depth. The volume of filtered seawater was calculated from the pump time and capacity. A mesh size of 150 µm is commonly applied in plankton surveys on sea lice abundance (Costelloe 1998, McKibben & Hay 2004, Penston et al. 2004, 2008, 2011, Molinet et al. 2011).

For the spatial survey, a fitted volume of 4.5 m\(^3\) was chosen, as previous studies inside fish cages suggested this to be sufficient (Costelloe et al. 1996, Gravil 1996). However, for the temporal study, the volume was increased to 6 m\(^3\), which was close to the maximum volume that is applicable in order to investigate samples at 3 depths every third hour, given the capacity of the plankton pump.

The intake of the pump was held at the selected depths by floats. Inside the net cages, samples were taken near the center of the 24 × 24 m cages while samples outside the cages were taken ~2 m outside the net (Fig. 1).

At distances from 40 to 340 m upstream and downstream the farm (Fig. 1), plankton samples were obtained with a 150 µm mesh size plankton net. The mouth diameter was 50 cm, and the uppermost part of the mouth was held at ~25 cm depth by floats and weights. The net was towed for 180 ± 10 m parallel to the fish cages by a fish farm catamaran at a constant speed of 0.8 m s\(^{-1}\). The location and length of the tows was recorded with a hand-held GPS (GPSmap 62s, Garmin). The feed pipes and feed barge positioned south of the farm, however, made it unfeasible to mirror the sampling distances north and south of the farm. Samples were preserved in ethanol (99.9%) and counted within 5 d after sampling. The entire sample content was analysed using a stereomicroscope, and the nauplii and copepodids were identified by their morphometrics and pigmentation pattern and colour (Schram 2004).

**Planktonic sea lice**

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**Sea lice on farmed fish**

Data on the farmed fish and on the abundance of adult *Lepeophtheirus salmonis* females and gravid *Caligus elongatus* present on the fish were provided by the fish farming company. The sea lice data was obtained from legislated sea lice monitoring, counting sea lice on 10 fish in each of 4 cages fortnightly. *L. salmonis* were grouped into preadults plus males and adult females with or without egg strings. *C. elongatus* were grouped into adults and gravid females.
Statistical analysis

The relationship between current speed and nauplii abundance at 1 m depth, and the nauplii depth distribution were investigated by general linear models with the statistical software package R (www.r-project.org). An exponential decrease in nauplii abundance as a function of depth was chosen, as the R² values of tested relationships suggested this to be the best fit. Thus, the nauplii abundance was log transformed prior to analysis by the general linear model. For the analysis of the nauplii abundance dependency on current speed, an invert linear relation with fixed intercept was used.

RESULTS

Hydrography

The current direction at 1 m depth at the fish farm was continuously northwards along the sound with the current speed changing with the semidiurnal tides (Fig. 2). In February the range in current speed measured approximately 240 m from the coast was 0.02 to 0.24 m s⁻¹, while in May the current measured ~70 m from the coast ranged from 0.02 to 0.12 m s⁻¹. Both records were conducted about 2 d before a neap tide of approximately the same strength. Thus, the difference in measured current speed is likely due to a gradient in current speed with distance from the coast.

Spatial distribution of sea lice

At the spatial survey in February, sea lice of the species *Lepeophtheirus salmonis* and *Caligus elongatus* were present in the plankton samples (Fig. 3). *C. elongatus* was the dominant species, with the *C. elongatus* to *L. salmonis* ratio being 2.7:1. The abundance of *L. salmonis* was low, and in the samples at the fish farm, the numbers ranged between 0 and 2 only. Thus, the sea lice abundance at the farm is presented as an average of the 4 cages (Fig. 3).

Nauplii dominated the planktonic stages with 95% of the observed sea lice. For both species, nauplii occurred sporadically upstream of the farm, while highest density was found within 125 m downstream (Fig. 3). The maximum *L. salmonis* density of 0.37 ind. m⁻³ was 40 m downstream, and maximum *C. elongatus* density was 1.1 ind. m⁻³ observed 125 m downstream. At the station 340 m downstream of the farm, nauplii density of both species was <0.06 ind. m⁻³, although at least 2/3 of the tow was in the direct wake of the farm, as measured by the current direction. At the fish farm, the *L. salmonis* nauplii abundance varied between 0 and 0.4 ind. m⁻³ while the *C. elongatus* abundance varied between 0 and 1.1 ind. m⁻³. In samples taken just outside the walkways of the platform, *L. salmonis* and *C. elongatus* nauplii were present in the 2 samples downstream of the cages, while no nauplii were found upstream.

Salmon lice copepodids were only observed in one sample upstream of the farm, and in a net cage. *C. elongatus* copepodids were observed in and around cages and in a sample downstream of the farm (Fig. 3).

The 24 h averaged surface water temperature was 6.3°C, and the average number of adult *L. salmonis* females per farmed fish was 1.6, while the average number of gravid *C. elongatus* was 2.7, as observed in the sea lice monitoring conducted 3 d after the present study.

Temporal abundance of sea lice

No planktonic *C. elongatus* were found during the temporal survey in May; all the sea lice were *L. salmonis*. As for the spatial survey, the bulk was nauplii (98%).
The density of *L. salmonis* nauplii in the investigated net cage was considerably higher than the density observed in the spatial survey. *L. salmonis* nauplii were present in all but one of the 25 samples, with densities of up to 4.2 nauplii m$^{-3}$ (Fig. 4). The average number of adult *L. salmonis* females on individual farmed fish was 1.3, which was somewhat lower than in the spatial survey, and the surface water temperature had increased to 7.8°C. The number of sea lice may vary between cages, and at the investigated farm the variation increased towards the end of the farming cycle as observed in sea lice monitoring data. Based on the between-cage variation in sea lice numbers in the monitoring programme, the number of adult *L. salmonis* females in the investigated fish cage ranged from 0.71 to 1.85 (95% confidence interval).

Copepodids were observed in 5 samples. The limited number of observations did not reveal any diurnal pattern in copepodid appearance as they were observed in 2 samples carried out in daylight and 3 during the night, nor was any vertical distribution pattern observed.

The depth-distributed nauplii abundance did not change with solar radiation (Fig. 4). The nauplii abundance rather showed a semidiurnal pattern, which was most pronounced at 1 m depth. The greatest variation, as well as highest mean nauplii density, was observed at this depth (Fig. 5). Mean nauplii abundance decreased exponentially with depth (Fig. 5). The exponential fitted plot ($R^2 = 0.9994$, $p < 0.05$) from the surface to the bottom of the cage shows a range in mean nauplii abundance from 2.5 naupl. m$^{-3}$ at the surface to 0.3 naupl. m$^{-3}$ at 10 m depth, and that 73% of the nauplii were located in the upper 6 m of the water column.

The semidiurnal variation in current speed (Fig. 2) and nauplii abundance (Fig. 4) indicates a dependency between the two, and there was an inverse relation between the current speed perpendicular to the cage and the nauplii abundance at 1 m depth (Fig. 6).

**DISCUSSION**

Observations of copepodids in association with the fish farm were scarce. They were absent from most samples, and maximum observed density was 0.3 ind. m$^{-3}$ for both *Lepeophtheirus salmonis* and...
Caligus elongatus. In a comparable study at a farm in Ireland (Costelloe et al. 1996), the same maximum density of L. salmonis copepodids was observed. Similar abundances of L. salmonis copepodids are generally observed in open water (Costelloe et al. 1996, Morton et al. 2011, á Norði et al. 2015). In the Loch Torridon system, sea lice abundance has been intensively studied at 5 open-water stations over several years, and the long time average density of copepodids at the stations was in the range of 0.01 to 0.6 copepodids (cop.) m\(^{-3}\) (Penston et al. 2004, 2011). Nearshore and in river mouths, copepodids can be much more abundant, and densities >100 cop. m\(^{-3}\) have been reported (McKibben & Hay 2004, Penston et al. 2004).

While the present study was not able to provide information on the vertical distribution of copepodids due to their low abundance in the samples, our results show that nauplii abundance decreased with depth but no diel vertical distribution pattern was present. This finding for nauplii is comparable to previous observations (Costelloe et al. 1996, Gravil 1996). The dominance of nauplii at the fish farm is also similar to previous observations (Costelloe et al. 1996, Morton et al. 2011, Penston et al. 2011), and in the present study the nauplii were highly abundant downstream of the farm, contrasting against the few nauplii found upstream (Fig. 3). Thus, the farm clearly acted as a source of L. salmonis at the end of the grow-out period.

Sea lice prevalence on farmed fish generally increases towards the end of the farming cycle (Torrissen et al. 2013) and, accordingly, the nauplii abundance around fish farms has been found to increase with age of the farmed fish in the Broughton Archipelago (Morton et al. 2011).

In the effort to mitigate the salmon lice infection pressure on farmed and wild fish, the finding that nauplii are much more concentrated at the farm than the copepodids might be useful. Mitigating the infection pressure in farming systems by targeting nauplii at the source, before they are distributed over wide areas by the currents, could be accomplished more easily than preventing copepodids from reaching farmed fish.

The spatial distribution of C. elongatus nauplii was similar to that of L. salmonis, although the abundance was considerably higher (Fig. 3). Thus, the farm was also a source of C. elongatus nauplii in February. The C. elongatus/L. salmonis nauplii ratio was 2.7:1, while the ratio between gravid C. elongatus and L. salmonis on the farmed fish was only 1.7:1. This could indicate that, at the time of sampling, the C. elongatus nauplii production was higher than the L. salmonis production, despite the number of eggs per string being considerably smaller in C. elongatus (Hogans & Trudeau 1989, Pike & Wadsworth 1999).
However, sea lice on wild fish aggregating around the farm (Dempster et al. 2009) might have contributed to the nauplii production.

In the temporal study conducted in May, *C. elongatus* were absent from the plankton samples. This is related to the seasonal pattern in *C. elongatus* abundance in the Faroe Islands, as they are present in high numbers during winter and virtually absent during late spring and summer (á Norði et al. 2015).

### Production of nauplii

The nauplii abundance inside a single fish cage varied considerably during the 24 h sampling period (Fig. 4). Greatest variation was observed at 1 m depth where the abundance was inversely related to the current speed at the same depth (Fig. 6). Costelloe et al. (1996) likewise observed a connection between nauplii density and current speed. In their study, current speeds below the measuring limit of 3 cm s⁻¹ were continuously recorded for 2–3 h, at which time the nauplii abundance in the cages was between 20 and 60 ind. m⁻³. At higher current speeds abundance ranged between 0 and 6 ind. m⁻³.

Assuming the nauplii production to be constant over time and that the nauplii remain at the same depth inside the cage, the flux of nauplii per area equals the total production per area upstream the measuring point

\[ P \cdot \Delta x = C \cdot u \]  

(1)

where *P* is the nauplii production per volume in the cage, \( \Delta x \) is the distance upstream the measuring point inside the cage, *C* is the nauplii concentration and *u* is the current speed perpendicular to the cage. Thus, the slope in the linear model presented in Fig. 6 provides the total production at 1 m depth upstream of the measuring point \( (P \cdot \Delta x = 0.072 \text{ nauplii m}^{-2} \text{ s}^{-1}) \). From this production rate, together with the amount of females upstream of the measuring point and the depth distribution of nauplii production, the *in situ* nauplii production was estimated to 26–68 nauplii female⁻¹ d⁻¹.

A number of assumptions and estimates were used for the above calculation. It is assumed that the fish, and hence the sea lice production, is evenly distributed horizontally in the cage, and although the sea lice abundance was only measured down to 6 m depth, a depth distribution down to the bottom of the 20 m deep cage is assumed. According to the nauplii depth distribution, 17% of the total nauplii production occurred at 1 m depth. It is also assumed that the distance from the outmost point of nauplii release to the measuring point (12 m) is too short for their behavioural traits to influence their depth distribution.

The depth distribution of nauplii inside the fish cages is dependent on the swimming depth of the fish, which varies in response to environmental factors as well as feeding pattern and artificial light. Darkness during nighttime and continuous feeding at the surface during the day are both drivers that draw the fish towards the surface (Oppedal et al. 2001, 2011), and the increasing temperature towards the surface in May (á Norði et al. 2015) likewise suggests that the fish had a vertical distribution towards the surface (Oppedal et al. 2011). This corresponds well with the observed vertical nauplii distribution in Fig. 5. The nauplii depth distribution is a major factor when estimating the production. Thus, the swimming depth of the farmed fish should be included in future production estimates.

The current speed, which influences the outflow of nauplii, is only measured at 1 m depth outside the net cage in this study. However, other current speed measurements in the area (K. Simonsen unpubl. data) have shown the speed at 0–20 m depth generally to be within the range measured at 1 m depth in this study, and that vertical changes are small. The net cages impose a drag which causes a reduction in water flow through the cages. The exact reduction depends on various factors such as current speed, mesh size and biofouling. Also the presence of the fish influences the flow through the cages and increases mixing (Klebert et al. 2013). On the other hand, the current at the study site increased with distance to the shore (Fig. 2), and the current measurement was conducted closer to the shore than the sea lice sampling (Fig. 1). Therefore, the measured current speed is assumed to represent the flow in the cage.

Most of the production estimates presented in the literature are in the range of 20–30 nauplii female⁻¹ d⁻¹, as obtained from the combination of the number of eggs in egg strings, hatching rate and development time. (Johnson & Albright 1991, Stien et al. 2005, Kristoffersen et al. 2014, Serra-Llinares et al. 2014, Johnsen et al. 2016). This corresponds to the lower end (26 nauplii female⁻¹ d⁻¹) of the estimated production range in the present study.

In conclusion, this study has demonstrated the farm as a source of salmon louse nauplii towards the end of the production cycle. Observations of the infective stage were scarce, and the study did not give insight into their origin. However, this study demonstrated a method to conduct *in situ* estimates of nauplii pro-
duction. Our estimates are in the same range, and therefore support, previous estimates of nauplii production that are based on laboratory trials, although the in situ estimate is associated with assumptions of unknown factors as discussed above. The production of nauplii only plays a partial role in the overall sea lice infection pressure, as during their planktonic stages, nauplii are subjected to various environmental conditions such as predation, risk of drifting to unsuitable environments, and failing to locate a suitable host. However, knowledge on the source and production rates is essential in the effort to mitigate infection pressure.

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