Optimal response to lousy circumstances:

The impact of salmon lice (*Lepeophtheirus salmonis*)

on depth preference of sea trout (*Salmo trutta*)

Thesis submitted in partial fulfilment of the requirements for the degree

Master of Science in Biodiversity, evolution and ecology

By

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FOREWORD

Due to ambitions of publication of this study in a scientific journal, this thesis is written to resemble an article manuscript. Hence, information and details that was not deemed crucial for communication of the main objectives were excluded from the main text and put into appendices.

ABSTRACT

Sea trout are known for seeking out sources of freshwater to rid themselves of salmon lice. Still, the effect of natural haloclines in fjords on parasite dynamics is not well understood. We tagged 48 wild caught sea trout, naturally infested by varying number of lice, with individual depth sensors. The fish were kept inside a small net-pen (4x4x5m) in Western Norway during four periods in spring 2017. The aim was to investigate how trout respond to salmon lice by changing their depth according to a natural halocline, and further elaborate on how this behaviour ultimately impacts their parasite abundance. The results show that temperature and light were the two most important factors explaining the vertical behaviour of trout. Mobile lice also had a significant effect on depth preference, where fish with higher abundances choose to swim shallower. However, individual variation in depth preference was larger than the impact of infestation levels, with some individuals choosing to stay deeper (and more saline) even though they had a high number of lice. There was a substantial reduction in salmon lice abundance during the seven days in the pen (68 ± 58 to 35 ± 18). The number of attached lice declined more rapidly when the temperature was high, most likely because of higher recruitment to mobile stages. Furthermore, the number of mobile lice showed a more substantial reduction when surface salinity was low. Surface salinity explained this reduction better than the experienced salinity of the individual. In summary, the results indicate that short-time exposure to very low salinities, rather than long-term exposure to moderate salinities, is the driving force behind the use of haloclines for delousing purposes.

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INTRODUCTION

Salmon lice (*Lepeophtheirus salmonis*) is an ectoparasite on salmonids, including sea trout (*Salmo trutta*) (Bjørn and Finstad, 1997), which may cause an osmotic and ionic imbalance in infected fish. This can be due both to mechanical damage, as well as a more general stress response in the fish (Bjørn and Finstad, 1997, Wells et al., 2006, Thorstad et al., 2015). Mechanical damage is caused by the lice grazing on the skin and mucous, leaving lesions and thereby damaging the natural barrier between the fish and the environment (Costello, 2006). In turn, this disturbance can lead to an increased loss of water and uptake of ions, causing osmotic and ionic imbalance, and in high intensities they can lead to death of the host (Bjørn and Finstad, 1997). Reduced levels of haematocrit have also been observed as an effect of this mechanical damage, due to bleedings and the shrinkage of the red blood cells due to dehydration (Wells et al., 2006)

Salmon lice have been, and continues to be, one of the most pressing issues in fish farming of Atlantic salmon in Norway and the Northern Hemisphere (Agnalt et al., 2017). While historically salmon lice were mostly a concern for the welfare and health of farmed salmon, it has grown to greatly affect wild conspecifics as well. Currently the effect of lice on wild trout and salmon is regulating the growth of the industry, through a recently ratified new set of policy rules of salmon farming in Norway ('traffic light system') (Vollset et al., 2017). The effect is quantified through risk assessments based on data from a national surveillance program, monitoring the level of salmon lice on wild populations of salmonids along the Norwegian coast (Taranger et al., 2015). Like in all monitoring, there are limitations to the data, concerning it being representative of the natural population. Data on the abundance of salmon lice on sea trout is currently being used as a proxy for the infestation pressure on wild salmon post-smolts, due to varying success and high costs related to methods for catching post-smolt (Taranger et al., 2015, Vollset et al., 2017). Still, the direct implications of this system on sea trout are uncertain as the knowledge around how populations respond to salmon lice is still short on documentation.

Usually, both smolt and adult individuals of sea trout emigrate from the river in early spring and return to the river either first, second or third autumn following the emigration, to spawn or overwinter (Thorstad et al., 2016). In areas with high infestation pressures of salmon lice, sea trout with high abundances of the parasite tend to return prematurely to freshwater, often

just days or weeks post their emigration from the river (Birkeland and Jakobsen, 1997, Thorstad et al., 2015). Although premature return relieves fish of salmon lice and osmoregulatory stress, and aids in shedding the lice, it also leads to loss of time at sea where fish can forage. This, in turn, affects the growth, leading to reduced fecundity and reproductive success (Birkeland, 1996).

Rivers are not the only possible source of freshwater for sea trout. The high input of freshwater in the inner parts of a fjord-system may cause a vertical stratification of the water, where lighter freshwater lays on top of the denser salt water. Sea trout can potentially exploit this top layer of freshwater, triggered by the same mechanisms that cause them to return prematurely. For instance, Helland et al. (2015) found that salmonid fish are less likely to have high infestation levels when freshwater run-off close to the sampling area was high. This indicates that the overall prevalence of freshwater in the fjord may influence the abundance of lice on wild salmonid fish. Recently, Halttunen et al. (2017) found that sea trout with acoustic transmitters positioned themselves in shallower waters and closer to the river in years when salmon lice infestation pressure from fish farms was high, with no difference between fish treated prophylactically against salmon lice and those not treated between years. A reasonable hypothesis, therefore, seems to be that salmon lice play a potential role in the vertical behaviour of sea trout.

Marine behaviour and vertical positioning of sea trout have been documented through previous studies (Rikardsen et al., 2007, Eldøy et al., 2017, Halttunen et al., 2017, Lyse et al., 1998). Equipment used in telemetry studies has improved significantly and become less expensive over the years. Studies from the 1980s and 1990s usually relied on small sample size, suffered from lack of individual-based data, and also fell short on accounting properly for environmental conditions (Lyse et al., 1998). In recent years several thorough investigations on sea trout behaviour (Eldøy et al., 2017, Halttunen et al., 2017, Rikardsen et al., 2007) have provided valuable insights into the response of sea trout to different environmental factors in natural conditions. However, a criticism of these studies is (1) that they lacked fine-scale sampling of the environmental factors and (2) could not determine the ultimate fate of the salmon lice present on the fish. Consequently, none of these does fully succeed in establishing an empirical link between observed behaviour and the cause of it. Also, these recent studies were carried out on a huge scale, resulting in a study design that is expensive and labour intensive, and thus hard to replicate.

The present project aimed to investigate the role of salmon lice as a factor in the optimal vertical positioning of sea trout at sea, and further elaborate on the ability of sea trout to compensate for the cost of salmon lice through fine-scaled adjustments in vertical behaviour. The study drew on individual high-frequency observations of 48 sea trout. Their behaviour was quantified by tracking their vertical positioning while kept inside a small net-pen (4x4x5m), making it the first telemetry study on the marine behaviour of wild sea trout in a semi-enclosed system. The depth data was subsequently linked to fine-scaled data on light intensity, salinity, temperature, the individual abundance of salmon lice, in addition to salmon lice related damage. Ultimately the goal was to improve monitoring data by better understanding the link between the observed salmon lice abundance on sea trout and the ultimate consequence of this infestation.

MATERIAL AND METHODS

STUDY AREA AND DESIGN

The general location of the current study was Herdlefjorden, a fjord located in the Askøy municipality in the county Hordaland, ca. 25 km northwest of Bergen (Norway). Hordaland is a county of high aquaculture intensity, with more than 120 active salmonid aquaculture facilities during the study period May and June 2017 (Norwegian Directorate of Fisheries). Aquaculture production along the Norwegian coastline is law regulated according to geographical production zones. Herdlefjorden is located in production zone 4 (PO4) (Nilsen et al., 2018). Results from the 2017 annual salmon lice surveillance on wild salmonids carried out by IMR show that PO4 came out with category "red", meaning that the estimated mortality in wild salmon due to salmon lice was over 30% in this area during the study period (Nilsen et al., 2018).

The specific location of the study was a sheltered bay in the northern part of Herdlefjorden, with a high level of freshwater impact due to 2 small river outlets nearby. The study was conducted in a net-pen measuring 4m×4m×5m. In each trial, the pen was stocked with 12 sea trout with surgically implanted depth sensors. Four sea trials were conducted between 2017.05.10 and 2017.06.25 and lasted 8-11 days (see Table 1 for details).

The data sampling was coordinated with the annual salmon lice surveillance program (NALO) at Herdla, and the project was applied for and approved by The Animal Research Authority (Application number 11838).

TABLE 1. Key environmental information for the four data periods

	Mean surface temperature (°C) $\pm$ SD	Mean surface salinity (ppt) $\pm$ SD	Mean light intensity ($\mu molS^2m^2$) $\pm$ SD
Period 1 2017.05.10 00:00:00 – 2017.05.17 23:00:00	11.2 ± 0.8	19.3 ± 3.5	375 ± 562
Period 2 2017.05.22 00:00:00 – 2017.06.01 23:00:00	14.1 ± 1.0	17.1 ± 1.7	517 ± 674
Period 3 2017.06.02 00:00:00 – 2017.06.12 23:00:00	13.6 ± 0.7	15.3 ± 1.7	308 ± 433
Period 4 2017.06.15 00:00:00 – 2017.06.25 23:00:00	14.3 ± 0.8	13.2 ± 2.3	336 ± 476

Note: For temperature and salinity, the data was sampled through the whole study period at 0.2m depth by the use of loggers. Light measurements were downloaded from a meteorological station owned by the geophysical institute at The University of Bergen located at Florida, Bergen.

FISH HANDLING

The fish were caught in 2 trap nets (Figure 1A in Appendix 2 for exact location, Figure 2A in Appendix 3 for illustration of trap net). Upon capture, individual fish were first anaesthetized (80 mg/L of Tricaine methanesulfonate (Finquel vet.™, Scan Aqua), measured for weight (g) and total length (mm) and examined for lice, and finally tagged (outlined below).

The salmon lice present were counted into the following categories; copepodid, chalimus 1, chalimus 2, preadult (no distinguishing of sex), adult males and adult females. Individuals of *Caligus elongatus* were also counted and registered. Skin damage associated with the lice was quantified by a scoring system (0-3, see Table 1A in Appendix 3).

A total of 48 sea trout (mean weight ± SD: 540g ± 328g, mean fork length: 385mm ± 61mm) were tagged with an acoustic tag (ADT-LP-7,3 from Thelmabiotel, Trans. Interval 30-90 sec, lifetime 150 days, 10 cm resolution, Figure 3A in Appendix 3). Seawater with a tricaine concentration of 40 mg/L was continuously flushed over the gills by the use of a silicone tube, to oxygenate the fish and to ensure that the fish was anesthetized through the whole procedure (Figure 4A, Appendix 3). A small incision was made mid-ventral on the fish by use of a sterile scalpel (Swann-Morton no 12) (Figure 4A in Appendix 3). A pre-sterilized (ethanol) tag was then inserted into the abdominal cavity and, the incision was closed with two monofilament non-absorbable sutures (EH7144H) together with tissue adhesive (Histoacryl). In the three last periods, a broad-spectrum antibiotic cream (Terramycin-Polymyxin B) was used on the tags to reduce the risk of potential bacterial infections in the wound. Detections from the tags were recorded by the use of six Vemco acoustic receivers (VR2W-69kHz) attached to the outside of the net pen framework. As we desired 12 fish with minimum variance in size, the fish sometimes had to be tagged over several days, due to insufficient catch in the trap nets. During transportation between the trap nets, the tagging location and the net pen, the fish were kept in a tub with constant supply of fresh water.

At the end of a sampling period, the fish were killed by an overdose of tricaine and a blow to the head. The lice abundance and damage, weight and length, were then carefully registered in an identical procedure to the one applied during tagging. Blood samples were also collected from each fish before the tag was retrieved for reuse in the following periods. Blood samples were taken from the caudal blood vessels and haematocrit (Hct) and leukocyte (Lct) determined, to be able to readily identify individuals compromised by leaky incisions (low Hct) and/or secondary infections (abnormal Lct).

QUANTIFICATION OF ABIOTIC FACTORS

Conductivity and temperature were logged continuously on 0.2m, 0.5m, 1m, 2m, 2.5m and 5m of depth from 11.05.2017 to 25.06.2017 by the use of 5 loggers from Solinst (Levelogger Edge 3003). The loggers were attached to a rope at fixed depth intervals and programmed to record with a time interval of 5 minutes. The rope was attached to the top frame of the net pen in one end and had an anchor attached in the other end. The data were linked to the time and depth of the individual registrations to estimate the conditions experienced by the fish. Conductivity data was converted into salinity by the use of the Marelac package (Soetaert et al., 2016) in R studio (RCoreTeam, 2014). The pressure was set to 0, as no data on pressure

was attained, and it also did not seem to serve as a major factor for the salinity when testing different values for the variable.

For light measurements, a light sensor logger from LI-COR Environmental (LI-1500) was used together with an underwater quantum sensor from the same producer (LI-192). Light intensity was measured at one-meter depth intervals (0m, 1m, 2m, 3m, 4m and 5m) at three different locations inside the net pen at the initiation of each new period, in three of the four periods (2017.06.23, 2017.06.04 and 2017.06.17). The quantum sensor was mounted to the 2009S Lowering Frame with the sensor facing upwards. The depth of each measurement was estimated by matching pre-measured tape markings with an interval of 1 meter on the attached rope with the water surface. The measurements were used to calculate the light attenuation constant (k) by use of the following equation:

$$k = \frac{-1}{D} \cdot \ln\left(\frac{I_D}{I_0}\right), \quad (1)$$

where D is a specific depth, I_D is the corresponding light intensity at this depth and I_0 represents surface light intensity. Calculations were done for each set of measurements (3 replications of 0-5m). The k from period 2 (k= 0.38) was excluded from further analysis, due to being very deviant from the k from period 3 and period 4 (respectively, k= 0.71 & k= 0.69).

The surface light intensity was logged continuously with intervals of 5 minutes from 2017.05.25 to 2017.06.25 (period 2 to period 4) at a location approximately 3.5 km in linear distance from the location of the net (due to lack of power outlet close to the net). The plan was to use this data, together with the mean k from period 3 and period 4, to model the light intensity at different depths by the following equation:

$$I_D = I_0 \cdot e^{-k \cdot D} \quad (2)$$

As surface light intensity was not sampled in period 1, data on light intensity was downloaded from a meteorological station owned by the geophysical institute at The University of Bergen (Været i Bergen; https://veret.gfi.uib.no/) located at Florida, Bergen,

with the intention to use this as a substitute for the lack of data from this period. Light data from the two sources was first plotted against each other, and co-variation was then tested by the use of a linear regression model. Through the plots, a slight inconsistency was detected, as it seemed that the local data on light observation was lagging the data from the city centre by 1-6 hours over the entire sampling period. A period of 17 days with a consistent shift was adjusted with one hour and tested against the data from Florida. When log-transformed the test gave r^2=0.763, which was found to be sufficient for fulfilling the aim of the study. There was also some light data lacking at the start of each period due to the ocean measurements for calculation of the rate of attenuation (k). As a result, it was decided to substitute the local data on light intensity in favour of the more consistent data from the University of Bergen.

DATA FILTERING

Before initiation of the study the tags were tested at water surface for accurate calibration. 10 out of 12 tags needed further calibration, which was done by subtracting the offset detected in the surface test from all sensor values. Calibrations were controlled by filtering detections for negative values. Nine tags (10, 11, 12, 13, 14, 15, 17, 18 & 21) had negative sensor values and were hence recalibrated by subtracting the equivalent positive value from the original offset value. No further range tests were carried out, as this was deemed unnecessary due to the size of the study area and the density of receivers.

A total of 851 230 detections of vertical positioning were registered and downloaded from 6 receivers individual through the full study period (2017.05.10 – 2017.06.25). Of these, 364 were discarded due to false transmitter identification codes (ID), resulting in 850 866 detections generated by the 48 tagged trout. A further 147 detections were removed due to lack of matching data (miscalculation of onset of temperature and salinity sampling). Disturbances of the study, like checking up on the net pen, were logged, and all data within the period of disturbance (8 176) (start of disturbance minus 15 min – end of disturbance plus 15 min) was flagged in the dataset and excluded from the analysis. Fish that died during the study period was flagged in the dataset (185 001 data points) and excluded from further analysis. The fish showed signs of stress-induced behaviour at the beginning of each period. By studying plots of individual behaviour, a recovery period of 2 days was decided on (Appendix 7, Figure 5-8A), and detections within this time period (149 482) were flagged in the dataset and excluded from further analysis. Five detections from tag ID 11 in period

4 were considered false detections, as the tag was not present in the study area at this point. These detections were also flagged and excluded from subsequent analysis. Two fish (13 & 15) from period 3 were also flagged (34 059) due to critically low Hct levels. Hence, after this filtering, a total of 473 996 detections with its associated biotic and abiotic sample values were left for statistical modelling and analysis.

DATA ANALYSIS

A linear mixed effect model (Gałecki and Burzykowski, 2013) was applied to model the depth use of sea trout. On the basis of *a priori* hypothesis (Appendix 1) on salinity being the main driving force in the potential relationship between depth and lice, it was discussed whether experienced salinity would be a better response factor for testing the hypothesis. Nevertheless, this was outweighed by the high resolution of the depth measurements compared to that of the salinity data, as the factors also are highly correlated. Individual fish was defined as random effects. Vertical positioning in terms of water depth (D_{it}) was log-transformed due to behavioural responses being skewed towards the surface with fewer observations of "dives" towards the lower part of the net pen. In this analysis mean lice per gram fish between first and second estimation (L_i), fish length (M_i), surface temperature (T_t^{up}; sampled at 0.2 m depth), surface salinity (S_t^{up} ; sampled at 0.2 m depth) and surface light (C_t) were included as explanatory variables. The linearity between the $logD_{it}$ and the four explanatory variables was explored using smooth spline plots. It was decided to log-transform L_i as it appeared to be a log-log relationship between depth use and lice. The same was done for M_i and C_t. The others were kept linear, as there were no clear patterns in the data. Temperature and salinity at other depths, or the difference between surface and bottom depth, were explored as explanatory variables, but could not be included due to strong correlation with the surface measurements.

Fine-scaled behaviour was explored by looking at histograms of changes in depth (delta) from one detection to the next. Out of the 473 996 detections, 462 848 of them showed a delta ≤ 1, meaning that the fish remained within a 1-meter interval of water depth in 97.7 per cent of all time intervals of the dataset. To synchronise the frequencies of variables, observations for vertical positioning were averaged across every 10 minutes to do observations on the finest scale according to the explanatory variables (i.e. light measurements had 10 minutes intervals) (33 144 data points). Since the observations were

auto-correlated (i.e. observation at one time step was strongly correlated to the observation at the next times step) a serial autocorrelation structure was explored (AR1). For model selection, the Aikake's Information Criterion (AIC) was then applied to compare this model to a variant with no lag structure. The AIC clearly suggested that a model including AR1 was superior in terms of general model quality. However, the autocorrelation in the current model did not decay as fast as the AR1 model. Thus, it was decided to explore more complex autoregressive-moving average (ARMA) functions with deeper lag structures. However, although more complex models did reduce AIC values, they did not influence on parameter estimates and their variance. Consequently, the simple AR1 model was selected. The full model was tested with the three different lice parameter L_i^{TOT}, L_i^{MOB} and L_i^{DAM}, and compared by the use of likelihood ratio test in R (RCoreTeam, 2014).

The model with the best fit became the target of further selection by using dredge function in the MuMin package (Barto'n, 2018) in R (RCoreTeam, 2014). The full model without any interactions was as follows:

$$logD_{it} = \beta_0 + \beta_1 logL_i^j + \beta_2 logM_i + \beta_3 T_t^{up} + \beta_4 S_t^{up} + \beta_5 logC_t + u_{it},$$

$$j = TOT,\ MOB,\ DAM \tag{3}$$

Further interactions, according to *a priori* hypotheses (Appendix 1), was tested by comparing AIC, again by the use of likelihood ratio tests. Equation (4) illustrates the full-fledged model including all hypothesised interactions.

$$logD_{it} = \beta_0 + \beta_1 logL_i^j + \beta_2 logM_i + \beta_3 T_t^{up} + \beta_4 S_t^{up} + \beta_5 logC_t$$

$$+\gamma_1 logL_i^j \cdot logM_i + \gamma_2 logL_i^j \cdot lnT_t^{UP} + \gamma_3 logL_i^j \cdot logS_t^{UP} + \gamma_4 logL_i^j \cdot logC_t + u_{it}. \tag{4}$$

Were D_{it}, L_{it}, M_i, T_t^{up}, S_t^{up} and C_t is explained in the text above, while *i* denotes variation in the factor across individuals and *t* denotes variation across time. Interactions are illustrated by the use of γ.

Change in lice abundance from initiation to termination of each period was modelled using a generalised linear model (glm) with gaussian distribution, predicting lice abundance at termination according to *a priori* decided parameters (Appendix 1), by with data averaged across individual (n=35). Total lice abundance (L_i^{TOT2}), abundance of mobile stages (L_i^{MOB2}) and attached stages (L_i^{ATT2}) at termination was modelled separately, and parameters included total lice at initiation (L_i^{TOT1}), mobile lice at initiation (L_i^{MOB1}), attached lice at initiation (L_i^{ATT1}), mean experienced salinity ($\overline{S}_i^{EXP}$), mean experienced temperature ($\overline{T}_i^{EXP}$) and surface salinity ($\bar{S}_i^{UP}$). Due to not using lice per gram in this analysis weight was also included as a predictor (W_i). No interactions were included as we deemed the dataset insufficient. Full-fledged models are illustrated in equation 5, 6 and 7. Selection on all models (8, 9, 10) was done by the use of dredge function in R (RCoreTeam, 2014).

$$L_i^{TOT2} = \beta_0 + \beta_1^{TOT1} L_i^{TOT1} + \beta_2 W_i + \beta_3 \bar{S}_i^{UP} + \beta_4 \overline{S}_i^{EXP} + \beta_5 \overline{T}_i^{EXP} + u_i \quad (5)$$

$$L_i^{MOB2} = \beta_0 + \beta_1^{MOB1} L_i^{MOB1} + \beta_2 W_i + \beta_3 \bar{S}_i^{UP} + \beta_4 \overline{S}_i^{EXP} + \beta_5 \overline{T}_i^{EXP} + \beta_6^{ATT1} L_i^{ATT1} + u_i \quad (6)$$

$$L_i^{ATT2} = \beta_0 + \beta_1^{ATT1} L_i^{ATT1} + \beta_2 W_i + \beta_3 \bar{S}_i^{UP} + \beta_4 \overline{S}_i^{EXP} + \beta_5 \overline{T}_i^{EXP} + \beta_6^{MOB1} L_i^{MOB1} + u_i \quad (7)$$

There was no clear trend in the mortalities. For details, see Appendix 5.

RESULTS

TEMPERATURE AND SALINITY

The salinity decreased throughout the study from approximately 23 ppt to 15 ppt in the surface (0.2m) and 31 ppt to 28 ppt in the bottom (5m) (Figure 1 a). The temperature, on the other hand, increased from approximately 10 °C to 13°C in the surface (0.2m) and 9 °C ppt to 12 °C in the bottom (5 M) (Figure 1 b). Consequently, the range in salinity between the uppermost (0.2m) and the lowermost (5m) increased from 8 ppt to 13ppt (Figure 1 c)). For temperature, the range was highest (13.9 °C to 11.0°C) in period 2 and lowest for period 1 (11.1°C to 9.5 °C) (Figure 1 c).

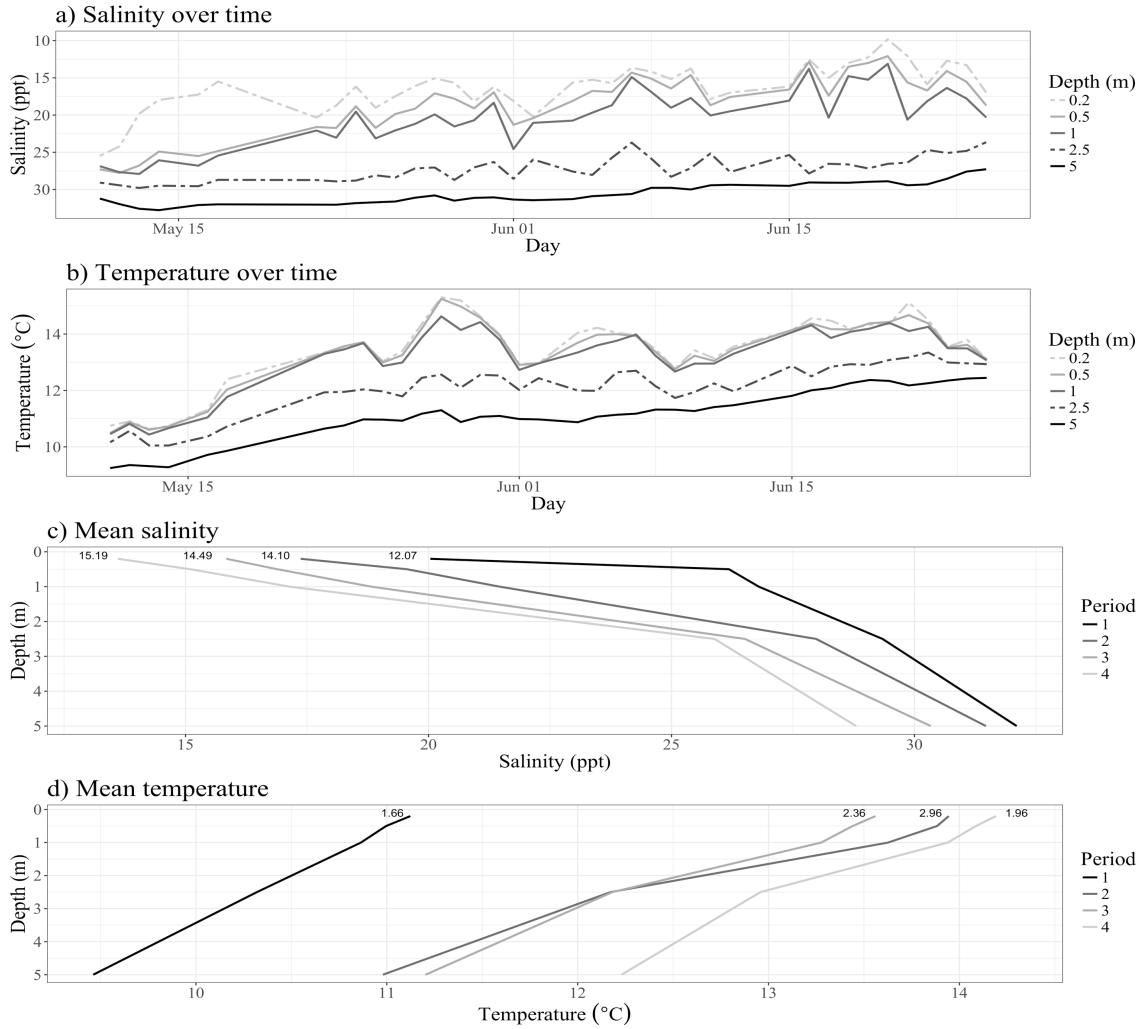

FIGURE 1 – a) and b) shows mean daily salinity and temperature at 0.2 – 5m depth throughout the whole study period (10.05.2017-25.06.2017), while c) and d) shows depth plotted against mean salinity and temperature for each of the 4 study periods. Annotation in c) and d) represents the range between the uppermost (0.2m) and the lowermost (5m) sample depth for each period.

DEPTH PREFERENCE

Fish that died exhibited a deviating vertical behaviour (Figure 2), probably explained by stress, as the same tendencies were seen at the beginning of each period (Figure 3 and 4). In period 3 the fish generally seemed less stressed, as less deviation at depth registrations were seen in the first 2 days (Appendix 7, Figure 7A). This was also the only period where none of the fish died during the experiment. This could not be linked to anything specific, as the methodology presumably was the same in this period compared to the rest. The observed data showed that the sea trout, in general, had a very narrow depth range, spending over 80 per cent of their time above 2.5 m. By plotting the depth registrations, it also became evident that sea trout exhibited diel migrations, staying slightly shallower during the night and deeper during the day (average depth 0.63m at night and 0.77 during the day; Figure 3).

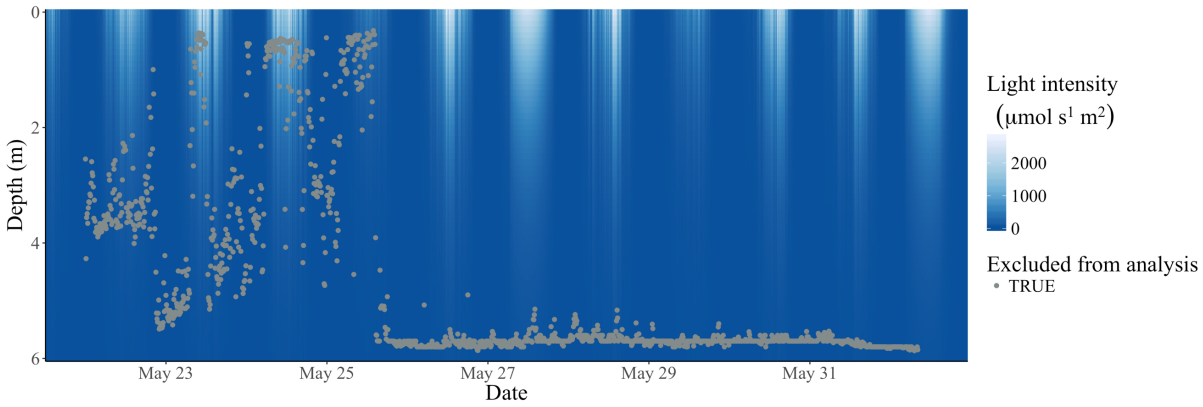

FIGURE 2 – Vertical distribution of fish 210 (ID 20, period 2, lenght=370mm, weight=393g, total of 10 mobile lice at the initiation of the experiment). All data excluded from the analysis due to mortality. The base layer in the plot represents light intensity at the associated depth.

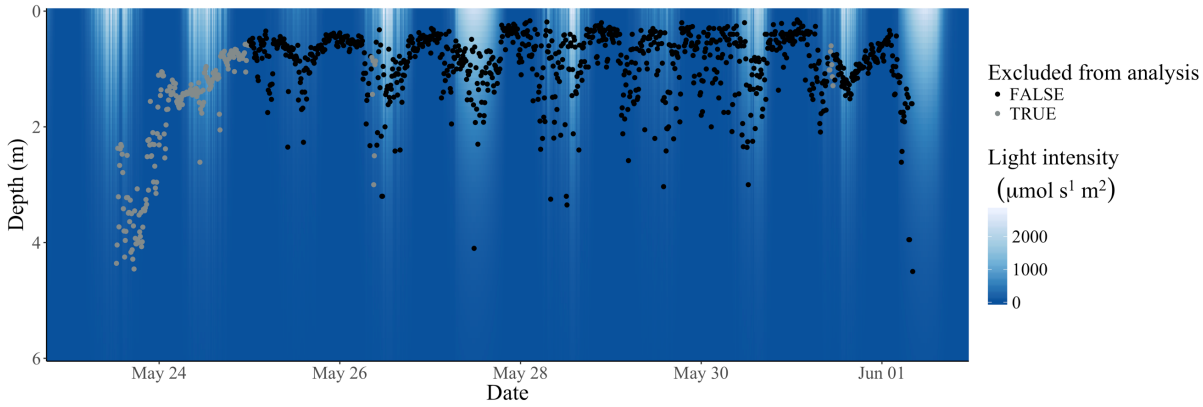

FIGURE 3 – Vertical distribution of fish 220 (ID 20, period 2, lenght=372mm, weight=434g, total of 1 mobile lice at the initiation of the experiment). Grey represents data excluded from the analysis either due to tagging effect or visiting of the study location. The base layer in the plot represents light intensity at the associated depth.

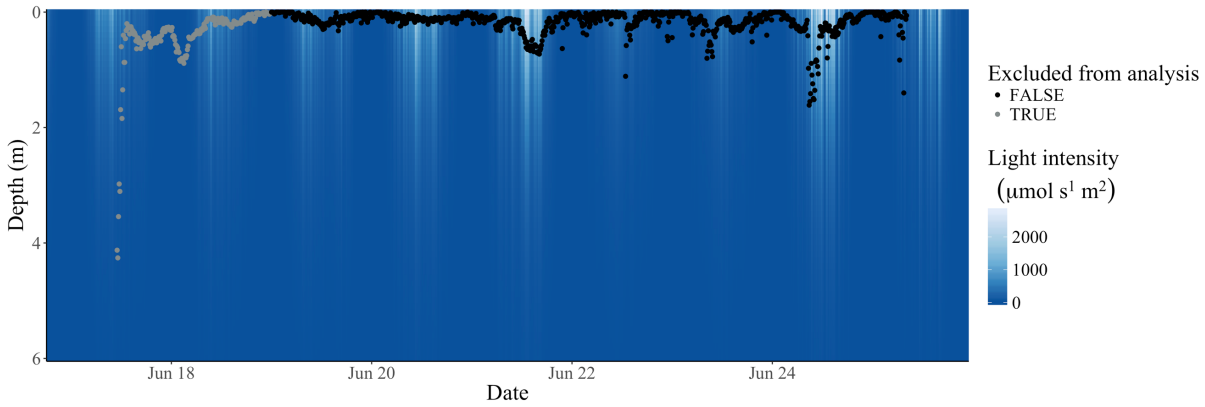

FIGURE 4 – Vertical distribution of fish 419 (ID 19, period 4, lenght=353mm, weight=464g, total of 32 mobile lice at the initiation of the experiment). Grey represents data excluded from the analysis either due to tagging effect or visiting of the study location. The base layer in the plot represents light intensity at the associated depth.

These observations were confirmed by the analysis, where all five of the best fitting models included surface light as a predictor for depth, in addition to temperature (Appendix 6, Table 5A). All three lice parameters (L_i^{TOT}, L_i^{MOB}, L_i^{DAM}) were examined separately, and mobile lice turned out to be the most significant predictor.

TABLE 2 - Top model from model selection 2 in Appendix 6 (Table 5A), where β_0 is intercept, C_t surface light intensity, T_t^{up} surface temperature (0.2m), S_t^{up} surface salinity (0.2m) and L_i^{MOB} is mobile lice per gram. *i* denotes variation in the factor across individuals and *t* denotes variation across time.

Top model, $logD_{it}$~	Estimate	p-value
Fixed effects		
β_0	0.363	0.052
$logC_t$	8.8e-6	0.012
T_t^{up}	-0.022	<0.001
$logL_i^{MOB}$	-0.134	0.033
S_t^{up}	0.003	0.135
Random effects		
SD - β_0	0.184	
Autocorrelation	Phi	
AR(1)	0.905	

According to the top model (Appendix 6, Table 5A, model selection 2 and Table 2) mobile lice (L_i^{MOB}, β_1= -0.134, p=0.033) and surface temperature (T_t^{up}, β_3= -0.022, p < 0.001) had a negative effect on depth. Hence, demonstrating that the fish occupied shallower depths with greater lice counts and temperature values, while surface light had a slight positive effect (C_t, $\beta_5 < 0.001$, p=0.012), meaning that the fish inhabited deeper habitat when light intensities were high. In addition, surface salinity (S_t^{up}, β_4= 0.003, p= 0.135) was included in the top model, despite not being significant (p-value>0.05). The parameter estimate was positive, meaning that fish swam shallower with low surface salinities.

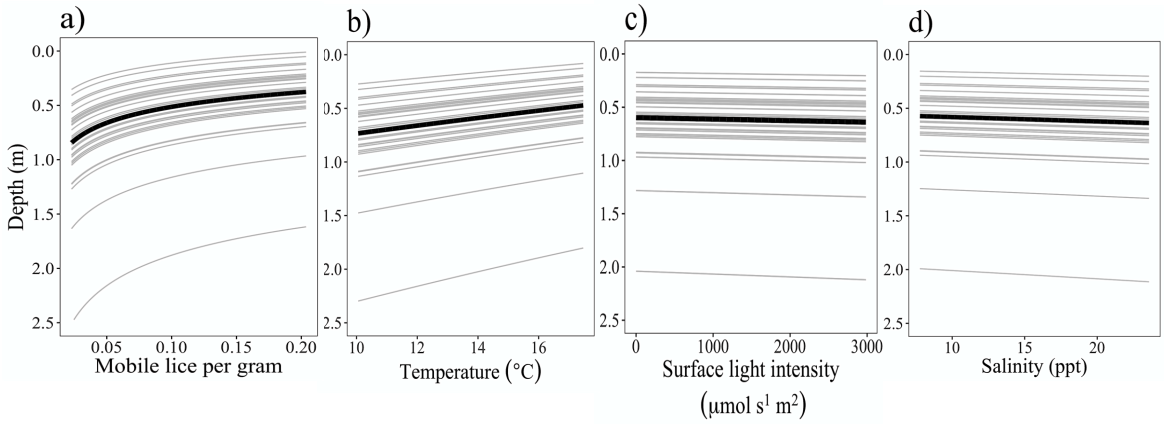

FIGURE 5 – Plots of predicted depth against the three parameters (a) L_i^{MOB}, b) S_t^{up}, c) T_t^{up}) included in the prefered model (Table 2). The remaining two parameters in each plot is set to the mean for the whole study period. The black line represents the mean of all individuals, while the grey lines illustrate the variance between the individuals. Depth has been retransformed from log to normal distribution

These results also coincided well with the raw data for both temperature and lice, but less for light, as there seemed to be a stronger relationship in the data than what showed up in the model (Figure 6). The same applied for the effect of surface salinity.

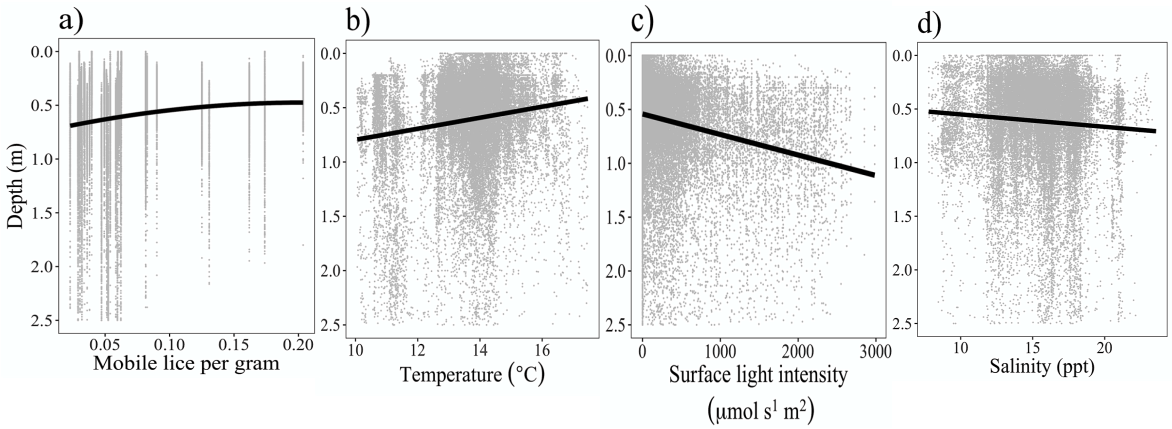

FIGURE 6 – Raw data of depth registrations (grey) plotted against the three parameters (a) L_i^{MOB}, b) S_t^{up}, c) T_t^{up}) included in the best fitting model for depth use (Table 2), fitted with a smoothing line (black).

A reason for this lack of pattern was most likely due to the most important component of the model being the random effect of individual, which was highly variable (Table 2), illustrated as the different grey lines in Figure 5. These results indicated that the different individuals overall choice played a bigger part in depth preference than the response to environmental factors. The consequence was a model that seemed to be less sensitive to environmental factors than what was expected after plotting of the observed data (Figure 6 and 7).

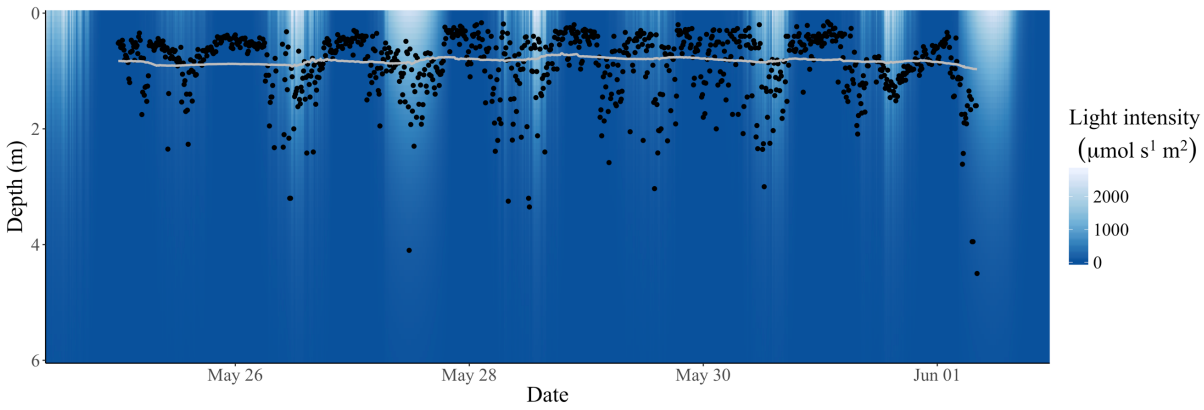

FIGURE 7 – Observed vertical distribution (black) of fish 220 (ID 20, period 2, length=372mm, weight=434g, total of 1 mobile lice at the initiation of the experiment), only with data included in analysis. Grey line represents the vertical distribution predicted by the chosen fitting model (Table 2) for the same individual. The data has been retransformed from log to normal distribution. The base layer in the plot represents light intensity at the associated depth.

Another critical point was also that the model seemingly did a poor job in predicting occurrences of sporadic dives into deep sections of the cage. To illustrate this the observed depth was compared with the depth predicted by the model using violin plots (Figure 8). However, the model did distinguish between the categories defined by the abundance of mobile lice showing that highly infected individuals stayed closer to the surface.

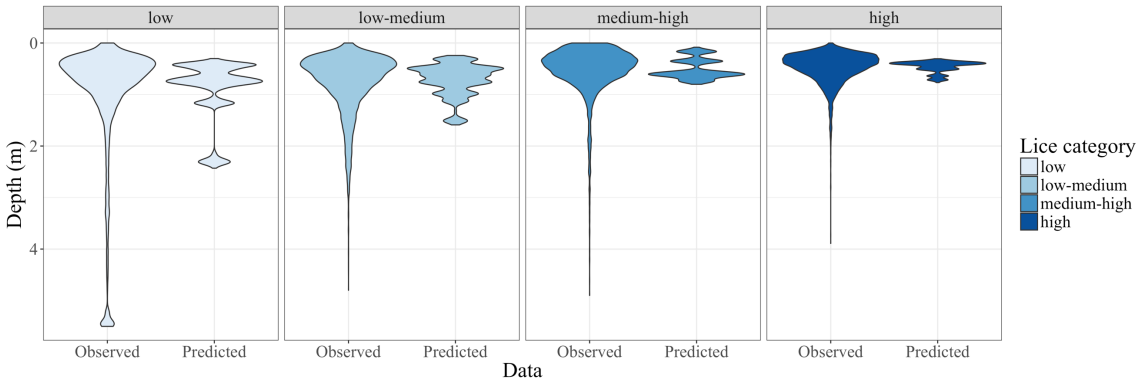

FIGURE 8 – Violin plots of observed versus predicted depth use from the top model (Table 2), retransformed from log to normal distribution, differentiated by category of mobile lice abundance. Categories were defined by the distribution of the data (min-Q1=”low”, Q1-Q2/median=”low-medium”, Q2/median-Q3=”medium-high”, Q3-max=”high”).

IMPACT ON LICE LEVEL

When looking at the mean data from each period, there seemed to be quite a dramatic change in lice level from start to end sampling during all the four study periods (Figure 9).

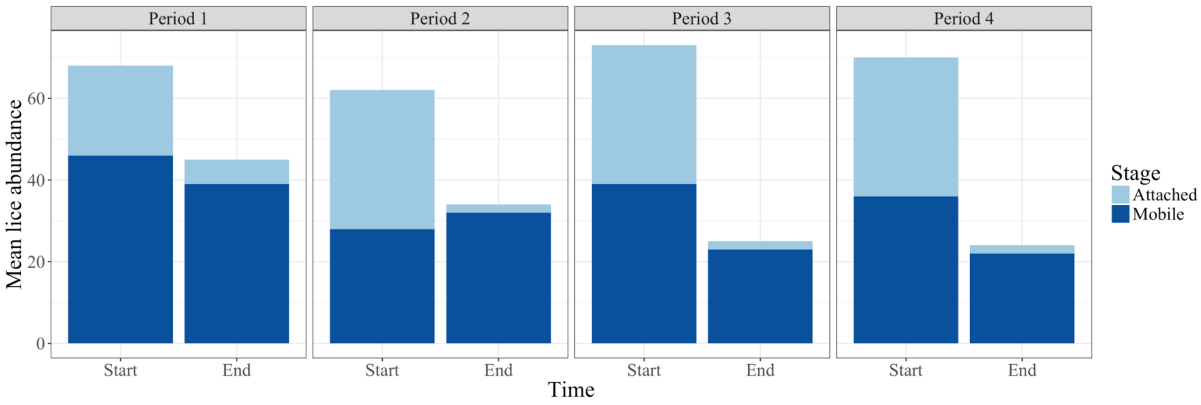

FIGURE 9 – Raw data of mean abundance of lice in each period at start and end.

Although not as prominent, the same pattern was found for each individual in our samples, as most fell beneath the 1:1 line in Figure 10 a), while only a few individuals recruited lice (i.e. was above the 1:1 line). The same applied when looking at just the mobile stages, with slightly more recruitment (Figure 10 b). For attached stages (Figure 10 c) all individuals fell beneath or on the 1:1 line.

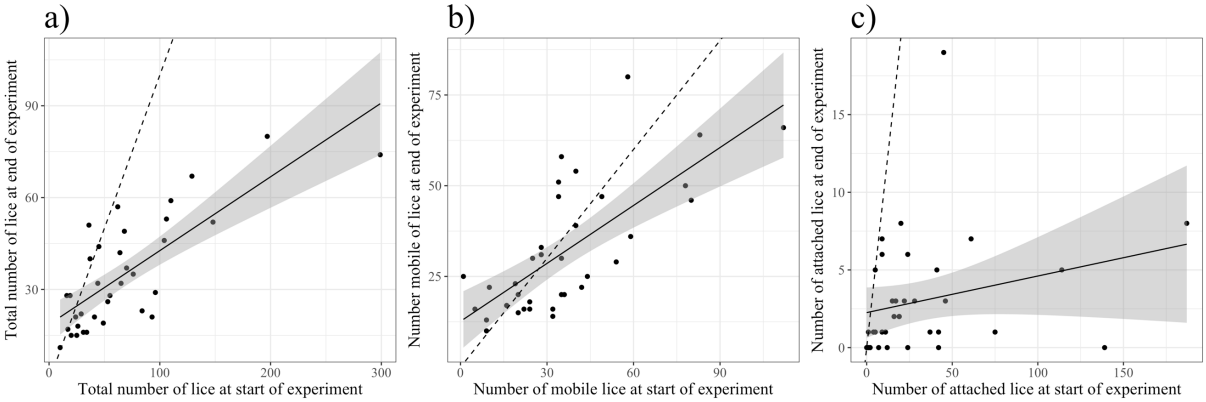

FIGURE 10 – Cross plot of end-of-period vs start-of-period lice abundance for all stages (a), mobile stages (b) and attached stages (c), fitted with a smoothing line and estimated confidence intervals. Dashed line represents 1:1line, hence no change from start to end.

When modelling end-of-period lice abundance, weight (W_i) turned up as a predictor in all five models, and for all stages of lice development (Appendix 6, Table 6A, model selection 3, 4 and 5). The parameter estimate was positive in all models, implying that higher weights

of the fish was associated with higher levels of lice. However, this predictor did not show up as significant in any of the top models (Table 3).

All the five best models in all selection sets included lice at start (Appendix 6, Table 6A, model selection 3, 4 and 5 a). For L_i^{TOT2} this was the total lice count at start (L_i^{TOT1}). The parameter estimates were positive and consistent in size in all of the five models, meaning more lice at start contributed to more lice at the end. When modelling for the end-of-period abundance of mobile lice (L_i^{MOB2}) the model included both the count of mobile (L_i^{MOB1}) and attached lice at the start (L_i^{ATT1}). Both parameter estimates were positive and consistent in size throughout all the models, but the estimate for mobile lice (L_i^{MOB1}) was more than twice the size of the one for attached lice at initiation (L_i^{ATT1}). Hence, mobile lice was the primary predictor, but attached also played a part, likely due to recruitment of attached lice into mobile stages with time. For the model selection for the end-of-period count of attached lice (L_i^{ATT2}), attached lice at the start (L_i^{ATT1}) was included in all five models, with parameter estimate that was positive and consistent in size throughout all models. Also, L_i^{MOB1} was included as a predictor in two out of the five models, including the top model. The estimate was small and negative, meaning lower values of L_i^{ATT2} when high values of L_i^{MOB1}.

Surface salinity ($\bar{S}_i^{UP}$) was included in three out of the five best models for L_i^{TOT2}, and in all five of the models for L_i^{MOB2}, including the top model in both model selection sets (p-value < 0.01) (Appendix 6, Table 6A, model selection 3 a) and 4). It was more or less consistent in size and positive in both cases, meaning higher values of L_i^{TOT2} and L_i^{MOB2} when surface salinity ($\bar{S}_i^{UP}$) was high, and vice versa (Figure 11).

Experienced temperature ($\bar{T}_i^{EXP}$) was included as a parameter in the model selection set for L_i^{TOT2} and L_i^{ATT2}. The parameter estimate is negative in both cases, showing that there were less lice at the end of the experiment when temperatures were high. For L_i^{ATT2} it was found in all five models, including the top model when strongly influential individuals (ID=115 and 414) were kept in the data (Appendix 6, Table 6A, model selection 5 a)). When removed, the predictor was excluded from three out of five models, including the top model (Appendix 6, Table 6A, model selection 5 b), and the whole model seemingly unravelled. For L_i^{TOT2}, $\bar{T}_i^{UP}$ was found in three out of the five best models, including the second-best model. The change in AIC (ΔAIC) from the top model was low, but including it changed the intercept

significantly (from -33.540 to 75.370). This could indicate lack of robustness or stability on parts of the model. As total lice abundance was a function of attached and mobile lice abundance, the effect of removing the influential individuals in the modelling of L_i^{TOT2} was explored. When doing so, surface salinity was excluded from the top model, while experienced temperature came up as a predictor in all models, including the top model (Appendix 6, Table 6A, model selection 3 b). In Figure 11 the effect of salinity on total and mobile lice, and the effect of temperature on attached lice is illustrated by residual plots (i.e. the effect of these factors when the other factors were corrected for).

TABLE 3 – Top model from model selection 3 a), 4 and 5 a) in Appendix 6 (Table 6A), where β_0 is intercept, $\bar{T}_i^{UP}$ averaged surface temperature (0.2m), $\bar{T}_i^{EXP}$ is averaged experienced temperature, $\bar{S}_i^{UP}$ averaged surface salinity (0.2m), W_i is mean weight of start and end sample, L_i^{TOT} is lice count including all stages, L_i^{MOB} is number of mobile lice and L_i^{ATT} is number of attached stages. *i* denotes variation in the factor across individuals and *t* denotes variation across time, while 1 and 2 denotes time of sampling (start=1, end=2).

Top model, $L_i^{TOT2} \sim$	Estimate	p-value
β_0	-36.686	0.007
L_i^{TOT1}	0.223	<0.001
$\bar{S}_i^{UP}$	3.345	<0.001
W_i	0.006	0.326
Top model, $L_i^{MOB2} \sim$		
β_0	-33.854	0.019
L_i^{ATT1}	0.142	0.015
L_i^{MOB1}	0.341	<0.001
$\bar{S}_i^{UP}$	2.845	0.002
W_i	0.007	0.310
Top model, $L_i^{ATT2} \sim$		
β_0	25.295	0.001
L_i^{ATT1}	0.052	0.008
L_i^{MOB1}	-0.046	0.136
$\overline{T}_i^{EXP}$	-1.660	0.004
W_i	-0.001	0.482

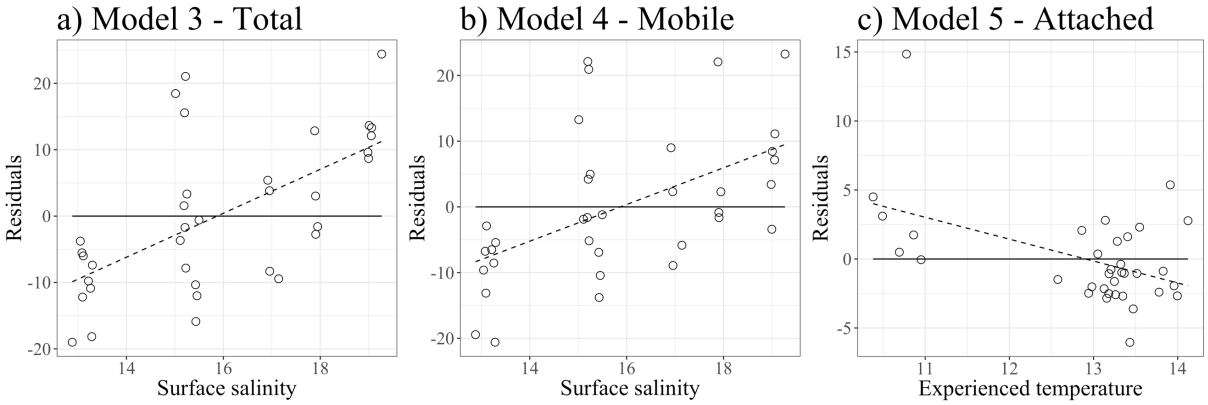

FIGURE 11 – Residual plots of the best fitting model (Table 3) for each of the 3 separate lice analyses, plotted against the environmental explanatory factor included in each mode. Points and dashed lines represent the dispersal of residuals when excluding the associated environmental explanatory factor.

DISCUSSION

The goal of this study was to document how sea trout behave according to vertical salinity profiles in the sea through vertical positioning as a response to osmotic stress caused by salmon lice. Sea trout in this study had a narrow depth range, but responded to surface temperature, light intensity and mobile stages of salmon lice, by swimming shallower with high temperatures, high abundances of mobile lice and low light intensities. However, individual variation in depth preference was much larger than the effect of infestation levels, with some individuals choosing to stay deeper (and more saline) even though they had a high number of lice.

A secondary goal was to investigate the change in lice on sea trout caught during salmon lice surveillance when being allowed to migrate vertically according to a natural halocline. The number of lice on sea trout generally decreased during the study period. After correcting for initial abundances of lice, it was clear that presence of surface salinity decreased the number of lice. As surface salinity explained this reduction better than the experienced salinity of the individual, it suggests that short exposure to very low salinities, rather than long-term exposure to moderate salinities, is the driving force behind the use of haloclines for delousing purposes. Also, temperature decreased the number of attached lice, most likely explained by the moulting of attached stages of lice into mobile stages.

The depth range found in the current study may not be entirely representative of real-life behaviour, when the vertical realm is larger. Still, our findings are reasonably consistent with findings from earlier studies (Lyse et al., 1998, Eldøy et al., 2017). Behavioural studies of sea trout in the marine environment, using telemetry, have shown that they primarily occupy the upper 1-5 m of the water column. However, there are very few studies on depth preference on sea trout (Thorstad et al., 2016). An exception is the detailed study of depth use was carried out in the Alta Fjord in 2007 using data storage tags (DST) (Rikardsen et al., 2007). They found that the tagged sea trout (body length 37-59 cm) occupied the upper 1-2 m of the surface more than half of their time, and in 90% of their time they were within the first 3 m. These results are generally in line with the findings of this study. As we did, Rikardsen et al. (2007) also found that fish had a deviating behaviour in the period just after release, when entering the marine habitat. This was explained by the switch in habitat, from freshwater to saline, and individuals spending less than two days at sea before recapture was therefore removed from the analysis. They fail to mention the potential effect of tagging, which is the hypothesised explanation for the deviating behaviour seen in the two first days after release in the current study. Another possible explanation is that the fish generally is stressed when introduced to the net pen, implying that initial stress levels could be associated with other explanations in addition to the tagging. The behaviour was also recognised for a more extended period of time for the fish that died before the end of the project. The data from the current study is not sufficient to make any conclusions on the cause. Nevertheless, the effect seems to wear off after approximately two days for all the fish that survived (Appendix 7, Figure 5-8A).

Light intensity is an important factor when studying depth preference as light is the main limiting factor for vision, which most fish, including sea trout, are dependent on when locating food and avoiding predators (Bone and Moore, 2008). Good light conditions make for good foraging conditions as the food is easy to detect, both for the individual and for the predators. Hence, fish face a trade-off between feeding and predator avoidance (Magnhagen et al., 2008). A common solution to this problem is to only occupy the shallow waters with high light intensities when feeding and relocate at greater depths the rest of the time to minimise the risk of being eaten (Magnhagen et al., 2008). This is the driving mechanism behind the behaviour known as diel vertical migration, which was recognised when looking at the observed data. The pattern was less prominent when looking at the data predicted by the best-fitted model. There is reason to believe this is a result of underestimation by the

model, as sea trout are known to have a tendency to swim deeper during the day and shallower during the night. This is supported by results from a detailed study conducted by Rikardsen et al. (2007) and later confirmed by a recent study by Eldøy et al. (2017).

Several factors could explain the lack of prominence of this pattern in the current model, one being the overshadowing individual variation. Also, the data on surface light intensity was log transformed in the model. The relevance of this was tested by fitting the same model with no log transformation of surface light. The difference in parameter estimates and AIC was minimal, and hence the log transformation was deemed trivial in this context. Another explanation could be the fact that the depth data was log transformed as well. This was our solution used for dealing with the data being aggregated close to the surface and not having negative values, drawing on the methodological response to the same challenge in previous studies like Eldøy et al. (2017). This approach implies less emphasis on extreme values of the data distribution, the values that seemed to be highly correlated with high surface light intensities, and hence potentially remove the pattern from the data. The result was a model that seemingly did a poor job in predicting occurrences of sporadic dives into deep sections of the cage (Figure 8 and Figure 9A in Appendix 7).

The model did distinguish between the categories defined by the abundance of mobile lice, showing that more highly infested individuals tended to stay closer to the surface (Figure 8 and Figure 9A in Appendix 7). When looking at the observed data, the diving-behaviour that was hypothesised to be a response to light was less prominent in the highly infected individuals (Appendix 7, Figure 9A). Judging by the size of the parameter estimate (Table 2), lice abundance seemed to be the best predictor of depth preference. This influence of lice on depth preference should be interpreted as a minimum, as several factors may weaken the pattern through the analysis, including the individual variation and lack of controls. Salmon lice are known to be the trigger of premature return to rivers during summer months (Birkeland, 1996, Birkeland and Jakobsen, 1997). The phenomenon is interpreted as a behavioural adaptation strategy to accommodate the osmoregulatory stress caused by the salmon lice (Wells et al., 2007, Birkeland and Jakobsen, 1997, Birkeland, 1996). As there was a strong correlation between salinity and depth, with salinities increasing with depth, the active use of freshwater refuge in the surface could be the underlying cause for the negative relationship between depth and abundance of mobile lice.

As mentioned there is generally a cost related to staying close to the surface, in relation to predator avoidance. Hence, as lice made the fish locate themselves in this risky area of the vertical realm, there must have been a benefit involved that outweighed this cost. This benefit could simply be the relief of the osmoregulatory stress caused by the lice. It could also be a reduction in the number of lice, as surface salinity was found to have a significantly adverse effect on lice abundances, both when modelling for all stages and when only modelling for mobile stages. This coincides with earlier studies where fish caught in the inner part of fjord systems, with high freshwater impact, was found to have less lice than fish caught further out (Helland et al., 2015). The fact that surface salinity proved a better predictor than experienced salinity suggests that rather than long-term exposure to moderate salinities, short-term exposure to low salinities is the driving force behind the use of haloclines for delousing purposes. Hence, the lower the salinity, the higher the benefit. This could be the explanation for the inclusion of surface salinity in the model for depth, although not significant (p-value > 0.05), as all fish were infested to some degree. One might also expect an interaction between surface salinity and mobile lice abundance, and also between light intensity and lice, with surface salinity having a more substantial impact on depth preference of individuals with high abundances, and light intensity having less impact on individuals with high abundances. This was tested for, and the model without the interactions outperformed the one including interactions. Hence, the study failed to support the presence of any interactions. Limitations of the study design, such as an uneven distribution of individual lice abundance, a small sample of individuals with low lice values, could be possible explanations for the lack of interactions in the data. Also, the effect of tagging as an osmoregulatory stressor, and hence the cause of the relationship between depth and surface salinity, could not be ruled out due to lack of control fish without tags. The absence of an interaction between light and lice could also be due to the small parameter estimate of light in the model, for which potential reasons have already been discussed.

Mobile lice abundance turned out to be a better predictor of depth preference than the total abundance of all stages. The epidemiology of salmon lice could explain this. Osmotic and ionic imbalance in the form of an increase in plasma chloride levels, caused by lesions in the skin, has been observed already when the salmon lice reach the chalimus stage (Bjørn and Finstad, 1997), but even more dramatic effects are found when salmon lice reach the preadult and adult life stages (Bjørn and Finstad, 1997, Wells et al., 2006, Wells et al., 2007), which marks a dramatic increase in the virulence. The abundance of lice is also found to be

positively correlated with the severity of osmoregulatory stress, indicated by a rise in plasma chloride levels (Bjørn and Finstad, 1997). Hence, it is in line with earlier findings that mobile possibly stages had a more substantial impact on the physiology of the fish than attached stages.

Temperature can have a large effect on the physiological processes in animals, including fish. There have been several studies on the preferred temperature of trout, and the results are not coinciding. While Larsson (2005) concluded on 16 °C, Reynolds and Casterlin (1979) landed on a considerably lower temperature of 12.2 °C. Larsson pinpoints the difference in feeding regime as a possible reason for the conflicting results, as the fish in the study of Reynolds & Casterlin's study were not fed, in contrast to Larsson's fish. Rikardsen et al. (2007) found that the fish stayed deeper throughout the summer, as the temperatures increased. Their finding also included sea trout experiencing higher temperatures throughout the season, hence not adequately compensating for increased temperatures with vertical displacement. The statistical analyses included two-sample t-tests on the data averaged on the individual. Hence, it was not tested for a relationship between depth use and temperature. Nevertheless, based on the reported results, in the case of there being a relationship it is likely positive, which is conflicting with the findings in the current study.

Endothermic animals, like birds and mammals, use behavioural and physiological strategies to increase their body temperature when infected with a pathogen, a response known as fever (Reynolds et al., 1976). As fish are obligate poikilotherms (ectotherms), they are extra vulnerable to temperature fluxes in the environment (Elliott and Elliott, 2010). Poikilotherms can achieve temperature increase through behavioural means only, by choosing an appropriate environmental temperature (Boltaña et al., 2013). This response, known as behavioural fever, has been documented in fish as a response to both bacterial (Reynolds et al., 1976), viral (Boltaña et al., 2013) and parasitic infestation (Mohammed et al., 2016). Is has also been proven to increase the survival of the individuals exhibiting the behavioural response (Boltaña et al., 2013, Reynolds et al., 1976). Hence, the negative relationship between depth preference and temperature could be explained by behavioural fever, either due to lice or bacterial infections in the wounds. , The significance of an interaction between temperature and lice was therefore tested, but did not meet the statistical requirements of being included in our preferred model (p-value > 0.05). The lack of interaction in the data could be explained by the lack of control fish with no lice, as the threshold for response in

altered temperature preference may be far beneath the lice levels observed in this experiment. The vast individual variance, pointed out earlier, could also explain the negative pattern between swimming depth and surface temperature, as the fish in the first period may have had a natural preference for staying deeper, and the temperature just happened to be very low during this period. The experienced temperature was also included as a predictor for the modelling of the end-of-period abundance of attached stages, with a negative parameter estimate. This is most likely explained by recruitment into mobile stages, as the rate of developments is highly dependent on temperature (Pike and Wadsworth, 1999).

Experienced temperature was also included in the second-best model for all stages when including highly influential individuals (Appendix 6, Table 6A, model selection 3 a), and in all models when excluding them (Appendix 6, Table 6A, model selection 3 b), with a negative parameter estimate. Hence, one could suspect mortality to have been higher in the attached stages when the temperature was high, as moulting cannot explain this. The effect of high temperatures on mortality of salmon lice is not well documented. A study conducted by Boxaspen (2006) showed that the parasite was absent from Norwegian salmon farms when the water temperature was higher than 18 °C. The maximum measured temperature in the surface during the current study was 17.5 °C, and 10% of the surface temperature values are over 15 °C. This is quite high and could be a possible explanation for the relationship. Nevertheless, due to the limitations of both the analysis of the attached stages and all stages, this has to be regarded as an indication only.

Another interesting finding, which has to be treated with caution, was the inclusion of mobile lice with a negative parameter estimate in the top model for the abundance of attached stages. This could indicate that mobile lice actively remove attached stages from the fish. The hypothesis has been brought up before, including in relation to an experiment conducted by Per Jakobsen in 1997. The experiment was a lab-based experiment including six fish tanks with Atlantic salmon (*Salmo salar*), three weeks post smoltification. For four of the tanks, female adult lice were placed on each fish, tree days prior to copepodid infestation in two tanks and three days post in two tanks. The two last tanks were kept with no female lice. The results indicated a significant difference in both mortality and lice level when comparing the tanks with and without female adult lice present, while there was no significant difference between the two different treatments of tanks including female lice (Jakobsen, P.,

Unpublished data). The mechanisms behind this is not known, but a possible interpretation is that adult lice through this process may prolong host survival and therefore indirectly their own lifetime reproductive success (Per Jakobsen, personal communication, 29. May 2018) Although lack of sturdiness in the analysis, the results of the current study could provide some support for these findings.

Eldøy et al. (2017) found there to be a relationship between depth preference and size of the fish, measured by length. Size does matter in the relation to antipredator strategies, as a smaller body makes for an easier prey, hence increasing the cost of staying in shallow and risky waters. When choosing fish for this study, it was aimed at a minimum variation in fish size, as the goal of the study was best fulfilled when the fish did not deviate in baseline behaviour. Although, due to limited catches, fish of variable size had to be included in the study. Hence, length was also included in the global model for swimming depth, although excluded during the model selection (Appendix 6, Table 5A, model selection 2). This could indicate that the selectivity on size was sufficient, as the individual variation was accounted for as a random effect.

The size was a factor when assessing the abundance of lice, as weight was a consistent predictor in all models of lice abundance, in all three separate model selections (Appendix 6, Table 6A). The relationship between size and number of lice has been reported before, through the study conducted by Serra-Llinares et al. (2016), where it was found that length had a positive effect on the probability of the fish being infested with attached stages of salmon lice. The relationship is likely a result of size induced differences in behaviour, with larger fish swimming deeper, faster and migrating farther, hence encountering more lice and experiencing higher salinity levels than fish of smaller size. In addition, larger fish have had a longer growth phase in the marine habitat, contributing to a longer period of exposure to the parasite.

LIMITATIONS AND SOURCES OF ERROR

The tagging of the fish was done by two different surgeons, as the surgeon for period 2, 3 and 4 had to be taught the technique by studying the more experienced surgeon tagging all the fish in period 1. This may have introduced a bias between periods. However, no overall pattern in mortalities or stress behaviour could be seen between periods, indicating that this was not a critical bias.

When tagging the fish, it was assumed that it would only affect the behaviour of the fish to a minimal degree and that stress caused by the surgery would not interfere with the results. As mentioned, this is an assumption that is somewhat problematic, as the wounds inflicted on the fish might cause osmoregulatory stress in the same way as lice, and hence create a bias in our data. On the other hand, not tagging the fish was not an option due to being dependent on individual-based data. Measures were taken to account for this error. The wounds were closed with both sutures and a tissue adhesive, to minimise leakage and limit the potential effect. Also, for each of the fish, the two first days of observations were excluded from the analysis due to deviant behaviour, potentially related to the effect of tagging. Nevertheless, it has to be taken into account when interpreting the results.

The fish handling, in general, can also alter the behaviour through stress, in addition to causing mobile lice to fall off. Because of this, all handling was done as carefully as possible to reduce the impact to a minimum. Also, the estimation of salmon lice abundance prior to each period was done after tagging, as the tagging procedure involves high amounts of handling.

Keeping the fish enclosed can also be a source of error, as it limits the natural range in depth preference to the size of the enclosure. Nevertheless, it was the only way of answering one of the main aims of the study; elaborate on the ability of sea trout to compensate for the cost of salmon lice through fine-scaled changes in behaviour. The enclosure did not seem to be a major factor, as the general depth distribution of the fish in the current study coincided well with earlier findings from studies of fish in their natural habitat (Rikardsen et al., 2007, Eldøy et al., 2017, Lyse et al., 1998). Although, this limitation in the study design cannot be excluded entirely when interpreting the results. Also, keeping the fish in such near proximity to each other increase the risk of inter-host transmission (Hull et al., 1998), introducing a level of uncertainty to the modelling of lice at the end of the experiment.

As mentioned, the study did not include any controls, neither for tags nor lice. Since all fish were wild caught and hence naturally infested, the only way to alter the lice abundance was to remove the lice from the fish manually. As the sample size of each period was limited to 12 tags it was decided against this, as doing so would significantly decrease the strength of the statistical analysis, due to low sample size of fish with lice. The same applied for

including controls without tags, in addition to the challenge related to obtaining data from fish without tags. Hence, including controls was deemed unfeasible for the current study, and the results have to be interpreted in the light of this limitation.

IMPLICATIONS FOR THE FUTURE

Currently, data originating from monitoring programs are used in the status assessment of one of the most critical obstacles to the Norwegian government's ambition for a fivefold increase in annual output of the aquaculture industry (Agnalt et al., 2017, Guttormsen, 2015). Results from the current study suggest more emphasis needs to be put on the importance of individual variation in this context, as not addressing it or underestimating its significance has the potential of introducing a considerable source of error concerning the question of representation.

The role of sea trout in the new monitoring system is still uncertain as knowledge around how populations respond to salmon lice is still poorly documented. The current study presented strong indications that salmon lice had an impact on the individual depth preference of trout, which in turn had an effect on lice abundances. This suggests that they, to some degree, were able to compensate for the cost of salmon lice through this fine-scaled change in vertical behaviour when the conditions were right. The results are also relatively easy to implement, as they suggest that the potential for compensation in a system is mostly dependent on the availability of low salinity surface water.

Based on findings in the current study, caution is advised when treating data that originate from the first days after tagging, as the behaviour during these days was found to be deviating from the rest. Future research should also put more emphasis on the statistical analysis than what has been seen up until now. To our knowledge, this was also the first study on marine behaviour of sea trout that addressed autocorrelation, despite the presence of published work on studies based on the same type of data (Eldøy et al., 2017, Rikardsen et al., 2007, Halttunen et al., 2017). Accounting for autocorrelation is strongly encouraged, as doing so significantly reduced the patterns in the data, suggesting an overparameterization of models trying to predict reality, inflated p-values and hence increased likelihood of a type II error when neglecting it.

CONCLUSION

The current study was the first telemetry on the marine behaviour of wild sea trout in a semi enclosed-system. The results indicate that the sea trout behaved relatively naturally compared to other studies on sea trout in the wild, making it a good alternative for pilot studies and for testing hypotheses that need a high-frequency data resolution. The results support already present knowledge on vertical diurnal behaviour, enlighten the role of salmon lice in optimal vertical positioning and open for discussion on the implications on temperature and salinity preference. In addition, they suggest a potential for compensating for the cost of salmon lice, through changes in vertical behaviour, that is directly linked to the surface salinity of the system. The high significance of individual variation highlights the limitations of monitoring studies. This calls for efforts in data validation, to even out the asymmetry between the impact force of the data and the uncertainty related to it. Finally, we hope that the effort put into analysis in the current study can provide inspiration and guidance for future research on similar subjects and/or similar types of data.

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APPENDICES

APPENDIX 1

Hypotheses

Temperature and light were hypothesised to be the main predictors of depth, as light is the governing factor for both foraging and antipredator strategies (Bone and Moore, 2008, Magnhagen et al., 2008), while temperature, in general, has a big impact on physiological processes in fish (Reynolds et al., 1976, Boltaña et al., 2013). Size was also believed to be a ruling factor, as length was found significant when modelling marine depth use of sea trout by Eldøy et al. (2017). Further, it was hypothesised a slight negative relationship between depth preference and lice, with fish going shallower and consequently into less saline water with high lice abundances, as an adapted behavioural response for dealing with the osmoregulatory stress caused by the lice. Lice abundance was also thought to interact with all abovementioned environmental factors, as the physiological effect of lice is dependent on infestation level (Bjørn and Finstad, 1997).

Salinity was hypothesised to be the primary factor for reduction of lice, as salmon lice are known to be intolerant to freshwater. Attached stages were predicted to be affected the most, due to the results of Wright et al. (2016), where attached stages were found to be more vulnerable to low salinities than mobile life stages. Further, it was predicted that more attached stages would develop into mobile stages with high temperatures, as the temperature is known to be the ruling factor of development in salmon lice (Pike and Wadsworth, 1999).

APPENDIX 2

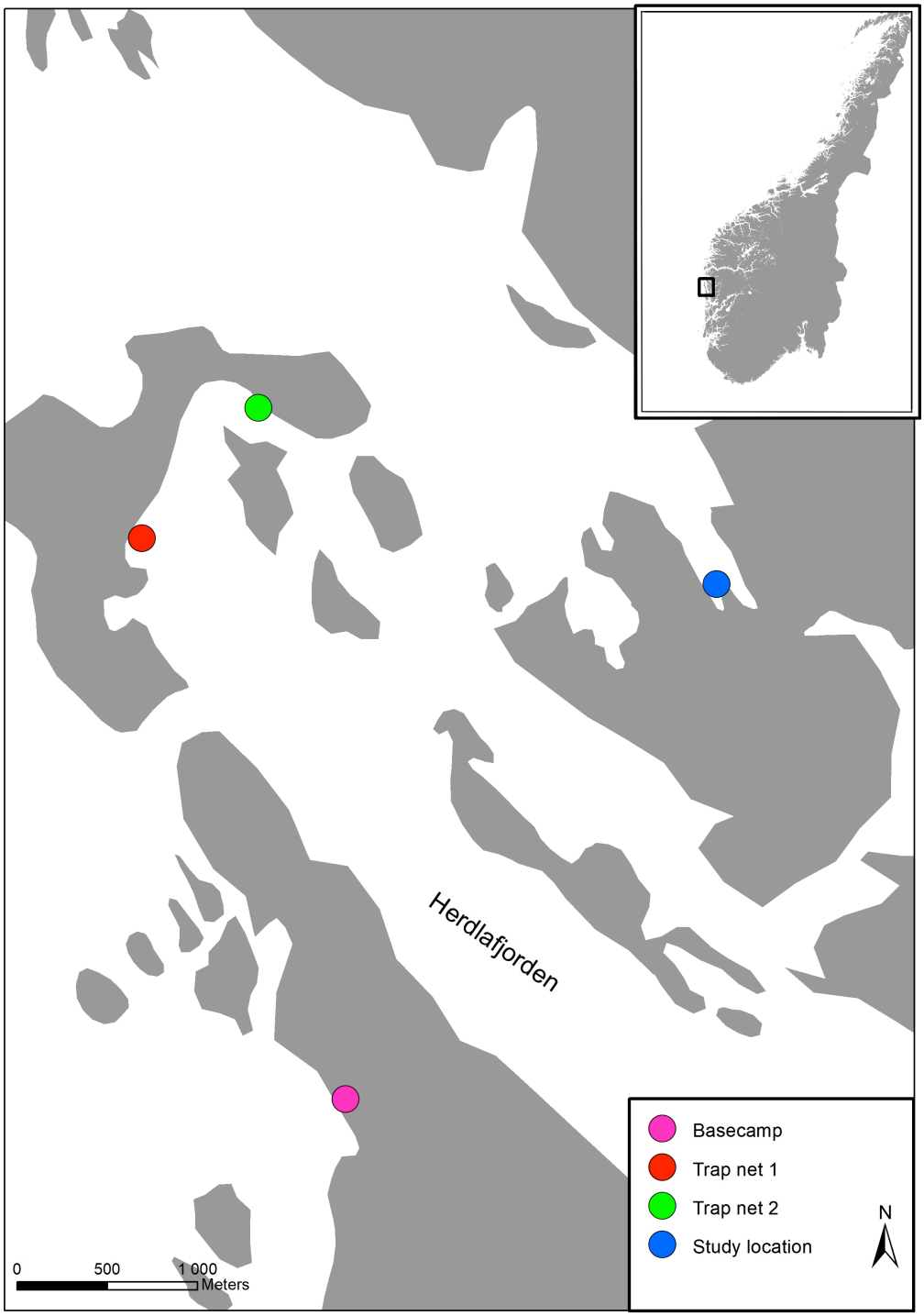
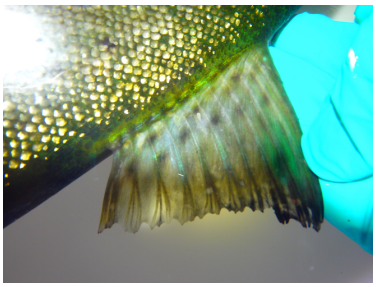
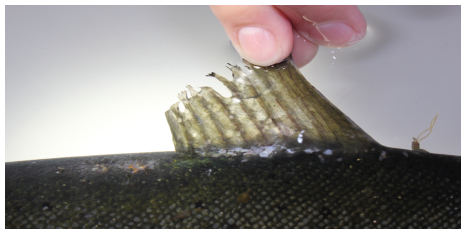
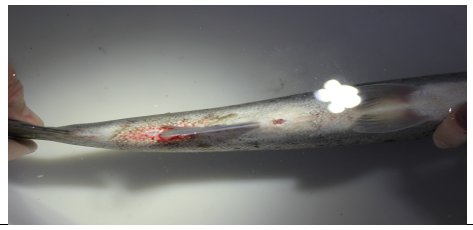
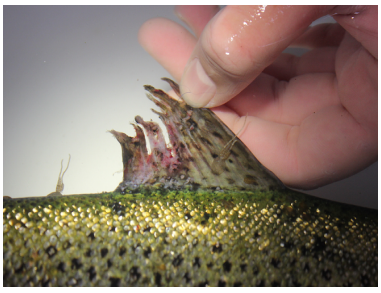
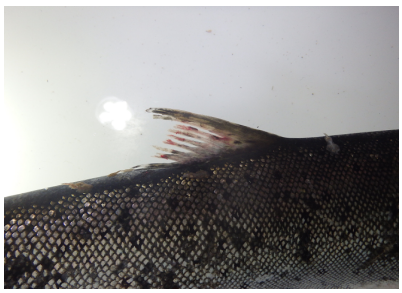
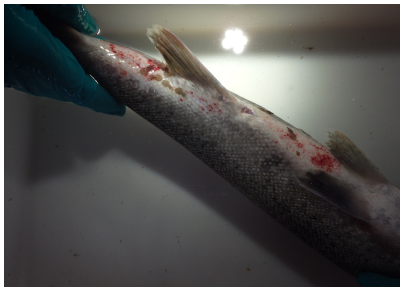


FIGURE 1A – Map of the study area. Trap net 1 and 2 represents the location of the two trap nets. Study location was the location of the net pen, and basecamp was the location where all fish handling was carried out.

APPENDIX 3

TABLE 1A – Score system for scoring of salmon lice related damage.

Level of damage	Score	Example
No visible damage	0	 
Light to moderate damage to dorsal fin OR visible lesions	1	
Light to moderate damage to dorsal fin AND visible lesions	2	
Moderate to severe damage to dorsal fin and moderate to severe case of lesions	3	   

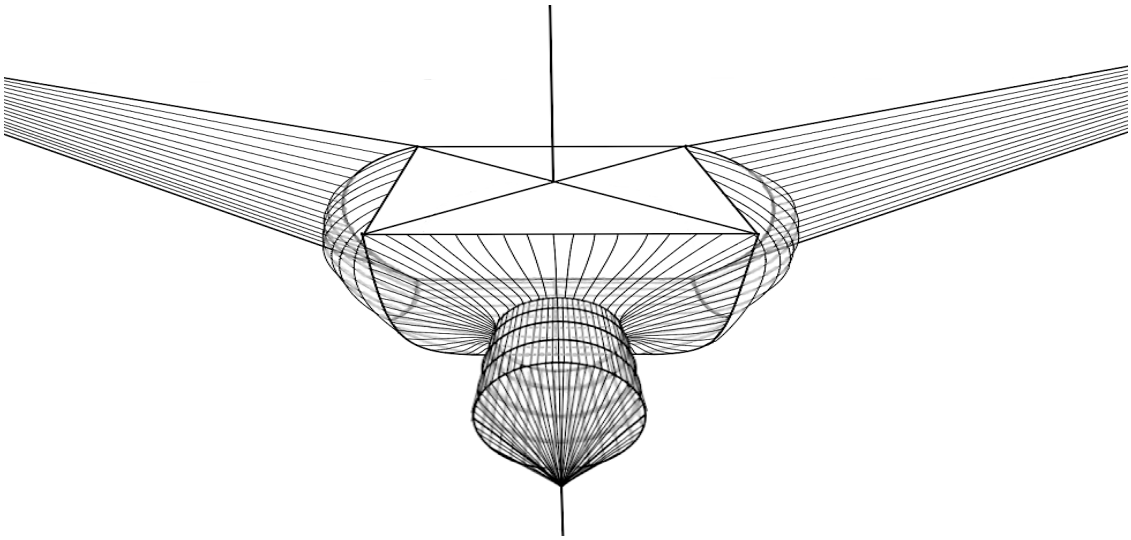


FIGURE 2A - Illustration on the design of the trap nets.

a) 

b) 

c) 

FIGURE 3A – a) The cage, b) Receiver, c) Tag.

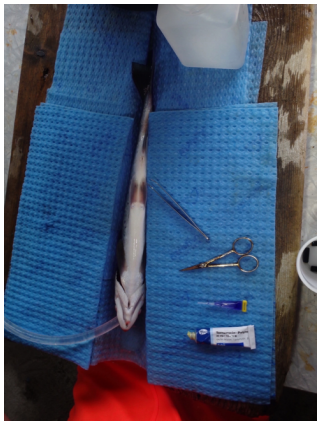




FIGURE 4A – Setup at the basecamp.

APPENDIX 4

Sea trout (*Salmo trutta*)

Brown trout is an adaptable species with a remarkable level of plasticity in relation to life history strategy, both within and across populations (Thorstad et al., 2016). Sea trout is a term for the individuals of brown trout that display an anadromous life history strategy (Thorstad et al., 2016). They spawn in freshwater, and the juveniles remain there for 1-8 years, during the life stage called parr. Parr are somewhat tolerant to saltwater but cannot tolerate full-strength seawater. Therefore, before undertaking their marine feeding migration, they undergo a series of physiological, morphological and behavioural changes; smoltification (Thorstad et al., 2016). In several behavioural studies, sea trout have been observed making rapid habitat changes in relation to salinity while in the marine habitat (Thorstad et al., 2016), suggesting that they can osmoregulate in low salinity waters even after the smoltification process without requiring acclimatisation.

Compared to salmon, there has not been much research on how sea trout populations are influenced by human activity, leaving sea trout among the least studied salmonid species (Thorstad et al., 2016). The sea trout has a life history strategy that differs from the Atlantic salmon. While the post-smolt of Atlantic salmon migrate far from its home river, the post-smolt of sea trout rarely travels farther than 80 km from the river of origin. It stays around in the fjords all summer, where the main sources of salmon lice, the fish farms, are also situated. Because of this, sea trout usually has much higher infestation levels than salmon (Hoddevik, 2017, Thorstad et al., 2015).

Osmoregulation in fish

Salinity is an abiotic factor that varies within both the horizontal and vertical realm (Wells, 2011). The factors that determine the salinity of the system are primarily evaporation and precipitation; rainfall and run-off. Both of these factors are governed by the climate and topography, resulting in a salinity distribution that varies between systems (Wells, 2011). Although, generally speaking, the salinity is lower at the surface and increases with depth, due to the chemical properties of freshwater and saltwater regarding density.

More than half of the current vertebrate species on earth are fishes, and they inhabit aqueous environments with widely ranging salinities, from freshwater to full strength seawater (McCormick et al., 2013). To cope with the challenges of each environment evolution has come up with several solutions to maintaining ionic and osmotic homeostasis (McCormick et al., 2013).

We distinguish between osmoconformers and osmoregulators; animals that are iso-osmotic to their environment and animals that either are hypo- or hyper-osmotic to their environment and expend energy on controlling uptake and loss of water and ions, respectively (Bone and Moore, 2008). All teleosts are osmoregulators that maintain a relatively narrow range of ionic concentration of 10-12 ‰ (ppt) in their body fluids (Stefansson et al., 2008), known state known as homeostasis.

Fish have large areas of thin and permeable epithelium in their epidermis, notably in the gills, which are specialised to make diffusion of oxygen and carbon dioxide as effective as possible (Bone and Moore, 2008). Naturally, these areas also serve as gateways for other compounds, like ions. The thin epithelium aids in the passive transport of ions along the osmotic gradient, moving towards equilibrium between the ionic concentration of the surrounding water and the body fluids of the fish (Bone and Moore, 2008). Hence, fish continuously have to expend energy on working against this natural osmotic gradient, actively transporting ions to compensate for the loss or gain of ions through osmosis. This active transport is very energy consuming and actually for as much as 25-50% of their total energy output (Bone and Moore, 2008).

Although all teleosts share approximately the same ionic concentration in their bodies, they do not share the same hassles in relation to osmoregulation, as their surrounding water has its distinct ionic concentration. Freshwater has an ionic concentration of < 3 ‰ (ppt), in contrast to saltwater with an ionic concentration of > 30 ‰ (ppt), making freshwater teleosts hyper-osmotic and marine teleosts hypo-osmotic to their environment (Bone and Moore, 2008). Hence, in line with the principles of osmosis, freshwater teleosts gain water and lose ions, while marine teleosts lose water and gain ions.

Salmon lice (*Lepeophtheirus salmonis*)

The salmon louse, or *Lepeophtheirus salmonis*, is a marine ectoparasitic copepod in the Caligidae family. It is a species specific parasite found on salmonid fish in both the North-Atlantic and the northern Pacific ocean, but they are regarded as two separate subspecies (Skern-Mauritzen et al., 2014).

The life cycle of the salmon louse consists of 8 stages separated by moulting; Nauplius 1 and 2 (planktonic), copepodid (infective stage), chalimus 1 and 2, pre-adult 1 and 2, and mature adult (Hamre et al., 2013). The infective copepodids attach themselves to the fish by a frontal filament and stay attached through the chalimus 1 and 2 stage, before losing the filament and becoming mobile from the first pre-adult stage (Costello, 2006). This represents a great shift in virulence, as the lice now are able to relocate on the fish, and hence cover a larger surface area. The lice feed on the mucous, tissue and blood of the fish, causing damage to the skin (Costello, 2006), hence making it prone to secondary infections and complicating osmoregulation.

The relationship between host and parasite is often characterised by a coevolutionary arms race (Bui et al., 2016), meaning they have existed simultaneously for many years. The salmon louse has always been a natural parasite on wild salmonid fishes in the sea. As a result, it has a life history strategy adapted for low densities of hosts, with short generation time and high fecundity. The females have internal fertilization and the fertilized eggs are pushed out, forming 2 strings extending from the abdomen. One single female can produce 11 pairs of egg strings over the timespan of a couple of months (Pike and Wadsworth, 1999). Each egg string containing up to 500, this makes for an enormous production of offspring during the lifespan of the lice (Pike and Wadsworth, 1999).

Aquaculture and salmon lice

The Norwegian salmon-farming industry was first established back in the 1960s. Shortly after the first aquaculture farms experienced epizootic outbreaks of the parasitic crustacean sea lice, *Lepeophtheirus salmonis,* better known as salmon lice (Pike and Wadsworth, 1999). The pattern became prominent not only in Norway but also in several other countries including Ireland and Scotland after the introduction of intensive salmon farming (Finstad et al., 2011).

The introduction of hosts at high densities year-round, provided excellent conditions for the parasite to flourish (Jansen et al., 2012, Torrissen et al., 2013). As the production of nauplii and copepodids rose, an increased infestation pressure could occur surrounding aquaculture farms. This situation was early recognized as a problem in the aquaculture industry, and later also for the wild salmonid fishes (Jansen et al., 2012).

Premature return

Premature return has been interpreted as a behavioural adaptation strategy to accommodate the osmoregulatory stress caused by the salmon lice (Birkeland, 1996, Birkeland and Jakobsen, 1997, Wells et al., 2007). The first documentation was done in Ireland in 1989-1991, when wild sea trout were observed to return prematurely to the rivers in a generally bad physical state, accompanied by high abundances of salmon lice (Whelan, 1991, Tully et al., 1993). Based on these findings, research on the topic was initiated in Norway, leading to the documentation of the phenomenon in Norway as well (Birkeland, 1996, Birkeland and Jakobsen, 1997).

As salmon lice are intolerant to freshwater (Finstad et al., 1995), over time the fish also rids itself of the lice by residing in freshwater. Birkeland (1996) concluded that the observed levels of salmon lice on the prematurely returning sea trout would most likely have resulted in death if the fish had not sought refuge in the river.

In laboratory experiments, it has been observed that the fish transferred to freshwater was more prone to secondary infections with bacteria or fungus (Wells et al., 2007). This has also been partly supported by data from the field as Birkeland (1996) found that almost 20% of the older migrants died within one week after return, although the specific cause of death was not determined.

APPENDIX 5

Survival analysis

A total of 10 fish died during the study; four in period 1, three in period 2 and three in period 4. Mortality was modelled using a glm (generalized linear model) with binomial distribution, and the data was averaged across individual. We wanted to investigate the significance of total lice density per gram (L_i^{TOT}), mobile lice per gram (L_i^{MOB}), and lice related damage (L_i^{DAM}). Weight (W_i) was also included, as there seemed to be a relationship between survival and weight when plotted against each other. As the dataset included fish that died during the experiment, the lice data used in the analysis was sampled at the start. For temperature and salinity, mean data available to each fish at 1m depth was used ($\overline{T}_i^{UP}$ and $\overline{S}_i^{UP}$, respectively). Further, 3 different models was fitted, due to strong correlation between several explanatory variables (in particular between salinity, water temperature and period (P_i)). All three models went through selection by replacing the original lice count variable L_i with L_i^{TOT}, L_i^{MOB} and L_i^{DAM}. The methodology of Burnham and Anderson (2002) was used to compare the resulting nine models. Further selection on the top model was carried out by the use of the dredge function in the MuMin package (Barto'n, 2018) in R (RCoreTeam, 2014). All models including damage were only fitted against the three last periods, as damage was not sampled in period 1.

$$H_i = \beta_0 + \beta_1^j log L_i^j + \beta_2 W_i + \beta_3 \bar{T}_i^{UP} + u_i,\ j = TOT,\ MOB,\ DAM \quad (1A)$$

$$H_i = \beta_0 + \beta_1^j log L_i^j + \beta_2 W_i + \beta_3 \bar{S}_i^{UP} + u_i,\ j = TOT,\ MOB,\ DAM \quad (2A)$$

$$H_i = \beta_0 + \beta_1^j log L_i^j + \beta_2 W_i + \beta_3 P_i + u_i,\ j = TOT,\ MOB,\ DAM \quad (3A)$$

Lice related damage (L_i^{DAM}) turned out to be the best lice parameter when compared to models including total lice abundance per gram (L_i^{TOT}) and mobile lice per gram (L_i^{MOB}) (Appendix 6, Table 4A, model selection 1 a). In this model selection temperature ($\bar{T}_i^{UP}$) proved to explain the data better than salinity ($\bar{S}_i^{UP}$) and period (P_i).

Hence, the model including L_i^{DAM}, W_i and $\bar{T}_i^{UP}$ became the target of further selection (Appendix 6, Table 4A, model selection 1 b). Top model of the model selection included W_i and period $\bar{T}_i^{UP}$ as parameters, although not significant (p-value>0.05).

TABLE 2A - Top model from Model selection 1 b) in Appendix 6 (Table 4A), excluding data from period 1.

Top model, H_i	Estimate	p-value
β_0	49.517	0.078
L_i^{DAM}	1.279	0.061
$\bar{T}_i^{UP}$	-3.630	0.078

The parameter estimate for L_i^{DAM} was positive, meaning that the model predicted a higher probability of survival when having high values of damage, while the estimate for $\bar{T}_i^{UP}$ is negative, meaning a lower probability of survival when temperatures were high (Table 2A).

As data from period 1 was not included in the analysis when using damage as a predictor, due to lack of data from this period, the potential of the second-best lice parameters, mobile lice per gram (L_i^{MOB}), was also further investigated (appendix 6, Table 4A, model selection 1 c)). The five best-fitted models for survival were very similar, concerning ΔAIC and AIC weights for model selection 1 c) (Appendix 6, Table 4A). The top model included W_i and period P_i as parameters, although not significant (p-value>0.05).

TABLE 3A - Top model from model selection 1 c) in Appendix 6 (Table 4A), fitted to the whole dataset. β_0 is intercept, W_i is start weight and P_i is period. *i* denotes variation in the factor across individuals and *t* denotes variation across time.

Top model,	Estimate	p-value
β_0	-0.890	0.378
W_i	0.003	0.142
$P_i(2)$	0.024	0.979
$P_i(3)$	18.160	0.992
$P_i(4)$	0.528	0.567

The parameter estimate for weight in the model was positive, meaning larger fish was more likely to survive than smaller fish. The estimate was small though, and the model just

including period came in as number three, with a ΔAIC of 0.59, and AIC weight of 0.150 versus 0.202 in the best model (Table 3A). This means that there was only a 5% difference in the likelihood of the top model being better than the one only explained by period. Hence, there seems to be something that was strongly correlated with period, that neither could be explained by salinity nor temperature. In period 2, 3 and 4 a broad-spectrum antibiotics cream was used in relation to the tagging procedure to prevent bacterial infections. It is unlikely that this is the variable in question, as the mortality in period 2 was almost the same as in period 1. Hence, the antibiotics did not seem to improve the survival of the fish in the current study. Due to the small differences in ΔAIC, and the fact that none of the predictors were significant in the model, the current study did not succeed in identifying a pattern in mortalities.

APPENDIX 6

TABLE 4A – Five best fitting models from the survival (H_i) analysis. β_0 is intercept, $\bar{T}_i^{UP}$ averaged temperature at 1m, $\bar{S}_i^{UP}$ averaged salinity at 1m, W_i is start weight, P_i is period, L_i^{TOT} is lice count including all stages, L_i^{MOB} is number of mobile lice and L_i^{DAM} is score of lice related damage according to Table 1A in Appendix 3. *i* denotes variation in the factor across individuals. AIC score is based on the Akaike´s information criterion (Burnham and Anderson, 2002), and ΔAIC is the amount of AIC that separates the model in question from the best fitting model (top model). Weight is considered the weight of evidence in favor of that specific model being the best fitting of the models included in the model selection, and is given as a probability estimate.

Model selection 1

a)		d.f.	Exp	AICc	ΔAICc	Weight
$H_i = \beta_0 + \beta_1^{DAM} logL_i^{DAM} + \beta_2 W_i + \beta_3 \bar{T}_i^{UP} + u_i$		5	1	41	0	0.498
$H_i = \beta_0 + \beta_1^{DAM} logL_i^{DAM} + \beta_2 W_i + \beta_3 P_i + u_i$		6	0.852	41	0.32	0.425
$H_i = \beta_0 + \beta_1^{DAM} logL_i^{DAM} + \beta_2 W_i + \beta_3 \bar{S}_i^{UP} + u_i$		5	0.15	45	3.79	0.075
$H_i = \beta_0 + \beta_1^{MOB} logL_i^{MOB} + \beta_2 W_i + \beta_3 P_i + u_i$		6	0.001	54	13.48	0.001
$H_i = \beta_0 + \beta_1^{TOT} logL_i^{TOT} + \beta_2 W_i + \beta_3 P_i + u_i$		6	0.001	54	13.75	0.001
$H_i = \beta_0 + \beta_1^{MOB} logL_i^{MOB} + \beta_2 W_i + \beta_3 \bar{S}_i^{UP} + u_i$		5	0.001	56	15.13	0
$H_i = \beta_0 + \beta_1^{MOB} logL_i^{MOB} + \beta_2 W_i + \beta_3 \bar{T}_i^{UP} + u_i$		5	0	56	15.52	0
$H_i = \beta_0 + \beta_1^{TOT} logL_i^{TOT} + \beta_2 W_i + \beta_3 \bar{S}_i^{UP} + u_i$		5	0	56	15.65	0
$H_i = \beta_0 + \beta_1^{TOT} logL_i^{TOT} + \beta_2 W_i + \beta_3 \bar{T}_i^{UP} + u_i$		5	0	58	16.85	0

b) - $H_i = \beta_0 + \beta_1^{DAM} logL_i^{DAM} + \beta_2 W_i + \beta_3 \bar{T}_i^{UP} + u_i$

	β_0	β_1^{DAM}	β_2	β_3	d.f.	logLik	AICc	ΔAICc	Weight
1	49.517	1.279		-3.630	3	-14.366	35	0	0.336
2	0.117	1.045			2	-16.309	37	1.5	0.159
3	34.121			-2.388	2	-16.561	37	2.01	0.123
4	1.421				1	-17.734	38	2.1	0.117
5	49.341	1.266	0.620	-3.619	4	-14.365	38	2.54	0.094

c) - $H_i = \beta_0 + \beta_1^{MOB} logL_i^{MOB} + \beta_2 W_i + \beta_3 P_i + u_i$

	β_0	β_1^{MOB}	β_2	β_3	d.f.	logLik	AICc	ΔAICc	Weight
1	-0.889		0.001	+	5	-20.991	53.4	0.00	0.202
2	2.333	0.885	0.003		3	-23.494	53.5	0.12	0.190
3	0.337			+	4	-22.537	54.0	0.59	0.150
4	0.873	0.667	0.001	+	6	-20.054	54.2	0.75	0.139
5	2.195	0.675		+	5	-21.581	54.6	1.18	0.112

TABLE 5A – Five best fitting models from the depth (D_{it}) analysis. β_0 is intercept, $\bar{T}_i^{UP}$ averaged surface temperature (0.2m), $\bar{S}_i^{UP}$ averaged surface salinity (0.2m), M_i is mean length of start and end sample and L_i^{MOB}. *i* denotes variation in the factor across individuals and *t* denotes variation across time. AIC score is based on the Akaike´s information criterion (Burnham and Anderson, 2002), and ΔAIC is the amount of AIC that separates the model in question from the best fitting model (top model). Weight is considered the weight of evidence in favor of that specific model being the best fitting of the models included in the model selection, and is given as a probability estimate.

Model selection 2 - $logD_{it} = \beta_0 + \beta_1 logL_i^{MOB} + \beta_2 logM_i + \beta_3 T_t^{up} + \beta_4 S_t^{up} + \beta_5 logC_t + u_{it}$.

	β_0	β_1	β_2	β_3	β_4	β_5	d.f.	logLik	AICc	ΔAICc	Weight
1	0.363	-0.134		-0.022	0.003	8.750e-06	8	27424.36	-54832.7	0.00	0.227
2	0.420	-0.133		-0.023		8.710e-06	7	27423.24	-54832.5	0.23	0.202
3	-0.709	-0.115	0.188	-0.022	0.003	8.753e-06	9	27424.67	-54831.3	1.38	0.114
4	-0.648	-0.114	0.188	-0.023		8.713e-06	8	27423.55	-54831.1	1.61	0.101
5	-1.337		0.348	-0.021	0.002	8.756e-06	8	27423.14	-54830.3	2.44	0.067

TABLE 6A – Model selection for lice analysis. β_0 is intercept, $\bar{T}_i^{EXP}$ is averaged experienced temperature, $\bar{S}_i^{UP}$ averaged surface salinity (0.2m), $\bar{S}_i^{EXP}$ averaged experienced salinity, W_i is mean weight of start and end sample, L_i^{TOT} is lice count including all stages, L_i^{MOB} is number of mobile lice and L_i^{ATT} is number of attached stages. *i* denotes variation in the factor across individuals and *t* denotes variation across time, while 1 and 2 denotes time of sampling (start=1, end=2). AIC score is based on the Akaike´s information criterion (Burnham and Anderson, 2002), and ΔAIC is the amount of AIC that separates the model in question from the best fitting model (top model)

Model selection 3 - $L_i^{TOT2} = \beta_0 + \beta_1^{TOT1} L_i^{TOT1} + \beta_2 W_i + \beta_3 \bar{S}_i^{UP} + \beta_4 \bar{S}_i^{EXP} + \beta_5 \bar{T}_i^{EXP} + u_i$

a)

	β_0	β_1^{TOT1}	β_2	β_3	β_4	β_5	d.f.	logLik	AICc	ΔAICc
1	-33.540	0.244	0.091	3.269			4	-126.619	262.6	0.00
2	75.370	0.198	0.073	3.937	-2.056	-6.233	7	-122.425	263.0	0.43
3	118.600	0.243	0.060			-7.281	5	-125.655	263.4	0.81
4	31.830	0.246	0.055				6	-124.282	263.6	0.99
5	66.980	0.245	0.045	3.016	-1.297	-5.386	6	-124.480	264.0	1.39

b)

1	111.900	0.263	0.149			-7.327	6	-114.968	245.2	0.00
2	95.460	0.310	0.075			-6.245	4	-118.548	246.5	1.36
3	92.670	0.242	0.071	3.027	-1.811	-7.118	7	-114.085	246.7	1.48
4	88.590	0.292	0.067			-6.029	5	-117.266	246.8	1.59
5	114.100	0.294	0.059			-7.237	5	-117.393	247.0	1.84

Model selection 4 - $L_i^{MOB2} = \beta_0 + \beta_1^{MOB1} L_i^{MOB1} + \beta_2 W_i + \beta_3 \bar{S}_i^{UP} + \beta_4 \bar{S}_i^{EXP} + \beta_5 \bar{T}_i^{EXP} + \beta_6^{ATT1} L_i^{ATT1} + u_i$

	β_0	β_1^{MOB1}	β_2	β_3	β_4	β_5	β_6^{ATT1}	d.f.	logLik	AICc	ΔAICc
1	-30.400	0.371	0.153	2.755			0.163	5	-128.565	269.2	0.00
2	-39.670	0.322	0.086	4.973	-1.419		0.111	7	-126.106	270.4	1.16
3	-28.570	0.361	0.070	2.895			0.161	6	-127.878	270.8	1.56
4	-31.980	0.375	0.069	3.887	-0.784		0.156	6	-127.902	270.8	1.60
5	-33.850	0.341	0.065	2.845			0.142	6	-127.954	270.9	1.71

Model selection 5 - $L_i^{ATT2} = \beta_0 + \beta_1^{ATT1} L_i^{ATT1} + \beta_2 W_i + \beta_3 \bar{S}_i^{UP} + \beta_4 \bar{S}_i^{EXP} + \beta_5 \bar{T}_i^{EXP} + \beta_6^{MOB1} L_i^{MOB1} + u_i$

a)

	β_0	β_1^{ATT1}	β_2	β_3	β_4	β_5	β_6^{MOB1}	d.f.	logLik	AICc	ΔAICc
1	24.860	0.047	0.069			-1.661	-0.053	4	-126.619	262.6	0.00
2	211.600	0.034	0.050		-1.963	-14.300		7	-122.425	263.0	0.64
3	21.500	0.029	0.043			-1.505		5	-125.655	263.4	0.95
4	44.820	0.050	0.041	-0.546		-2.534	-0.056	6	-124.282	263.6	1.06
5	267.200	0.040	0.040	-3.344	-1.859	-13.810		6	-124.480	264.0	1.08

b)

1	2.364		0.058					2	-75.677	155.8	0.00
2	10.370		0.056			-0.619		3	-74.492	155.8	0.06
3	-0.371		0.038		0.132			3	-74.887	156.6	0.85
4	12.280		0.029			-0.704	-0.023	4	-73.833	157.1	1.34
5	11.760		0.029			-0.655		4	-73.860	157.1	1.39

APPENDIX 7

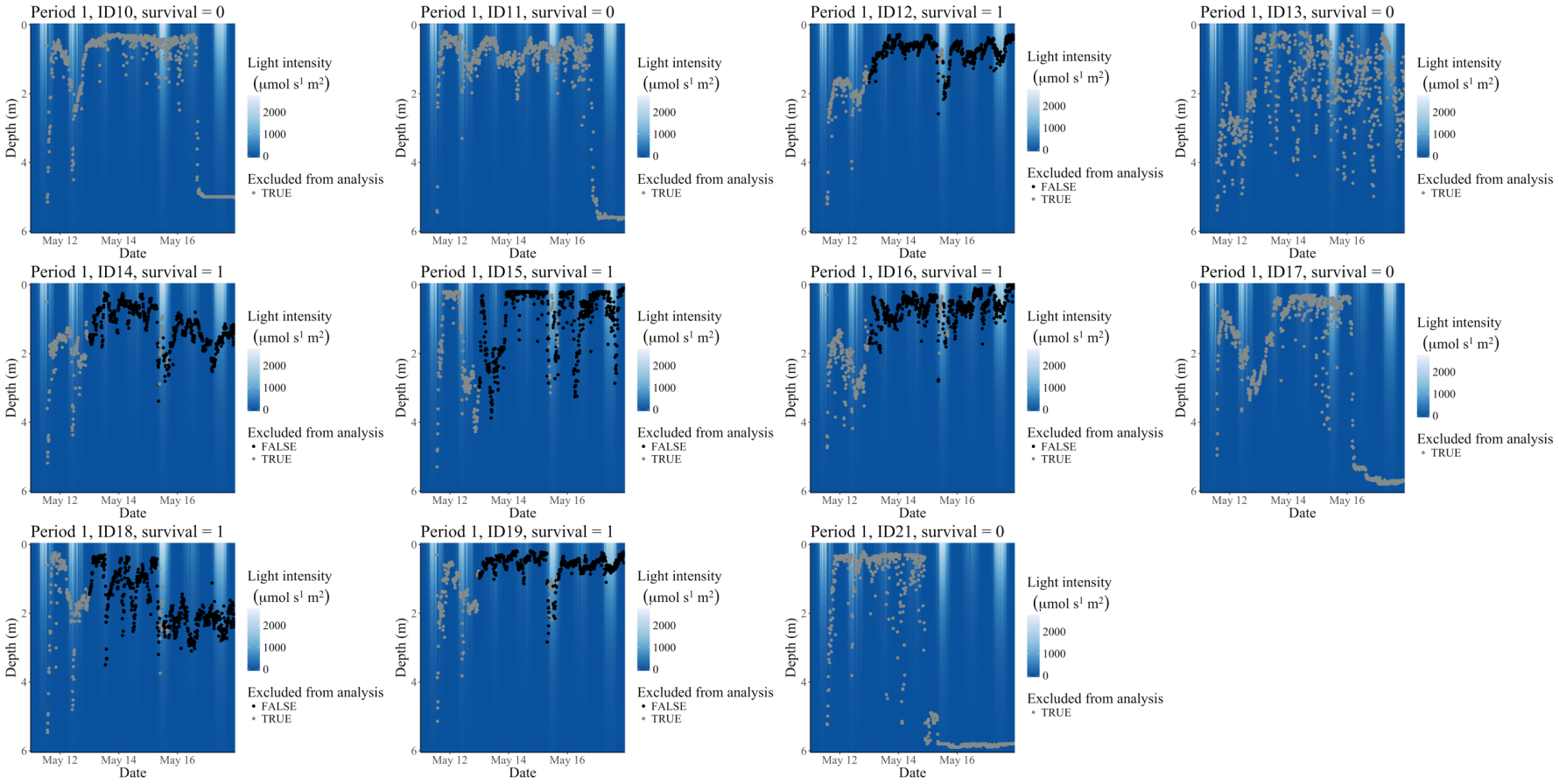


FIGURE 5A – Depth distribution of all individuals in period 1. We only got data on 11 individuals in this period, as there was one tag that was turned off during the experiment. Grey represents data excluded from the analysis either due to deviating behaviour or visiting of the study location. The base layer in the plot represents light intensity at the associated depth.

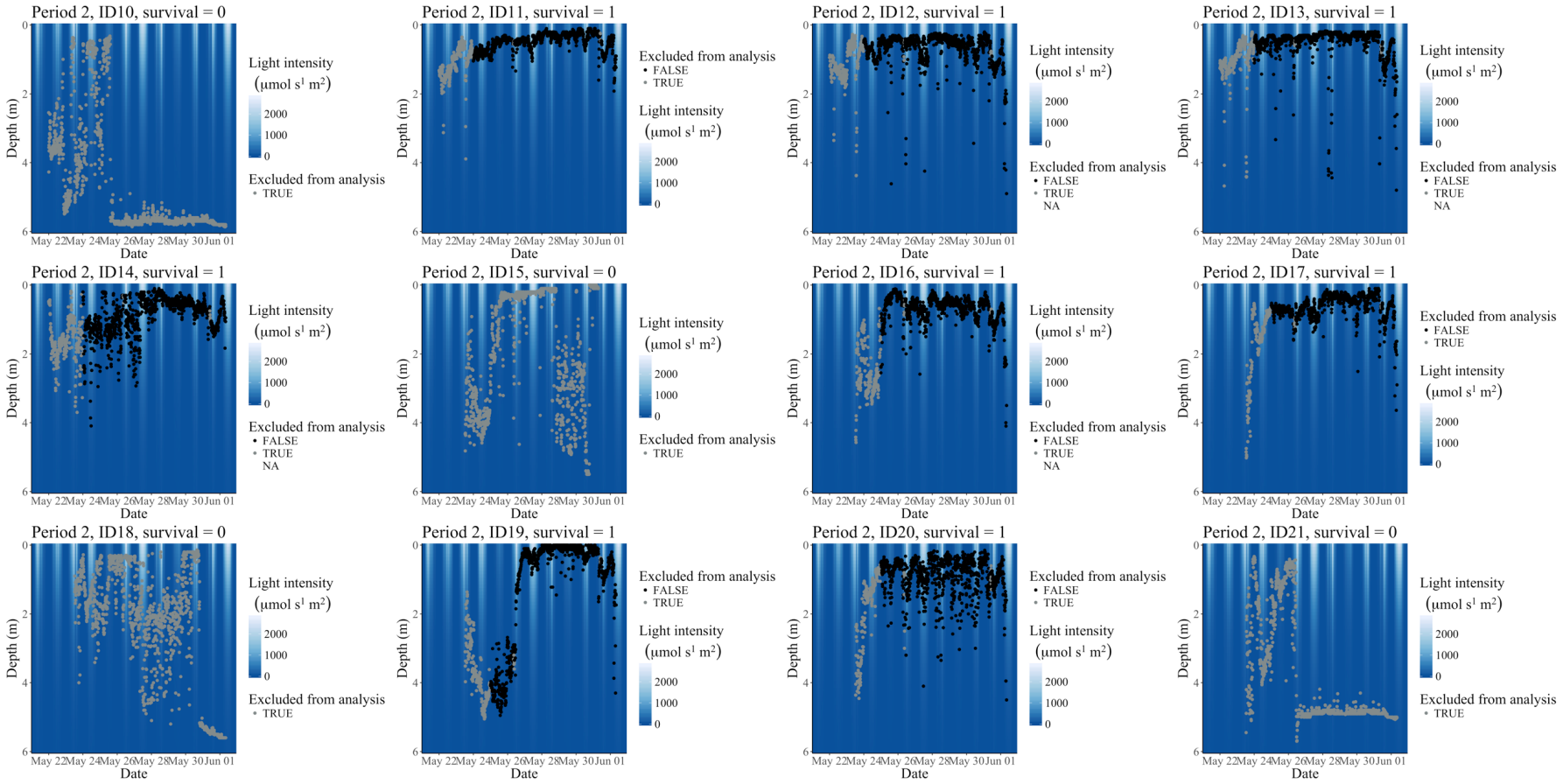


FIGURE 6A – Depth distribution of all individuals in period 2. Grey represents data excluded from the analysis either due to deviating behaviour or visiting of the study location. The base layer in the plot represents light intensity at the associated depth.

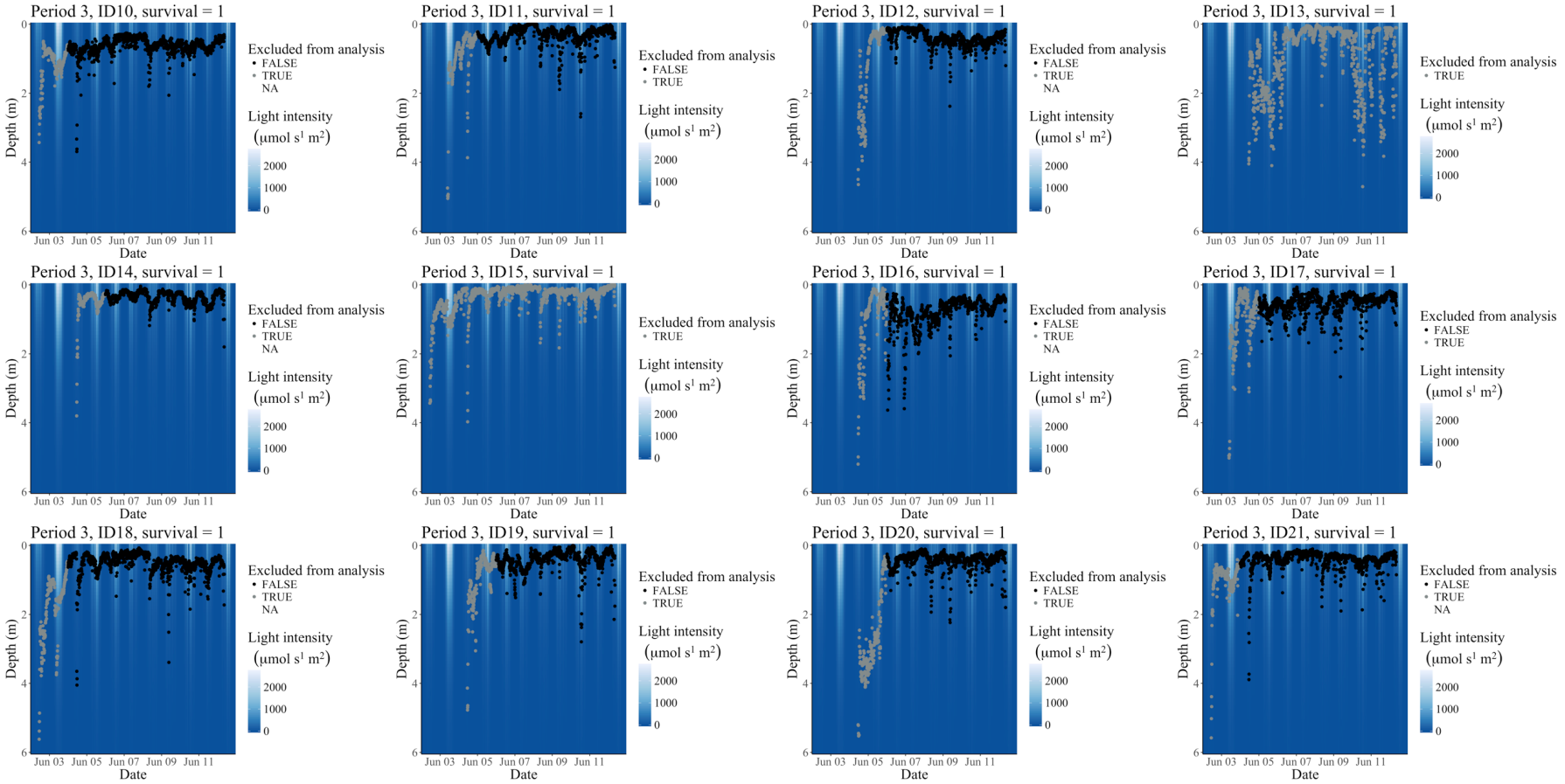


FIGURE 7A – Depth distribution of all individuals in period 3. Grey represents data excluded from the analysis either due to deviating behaviour, visiting of the study location or critically low haematocrit levels. The base layer in the plot represents light intensity at the associated depth.

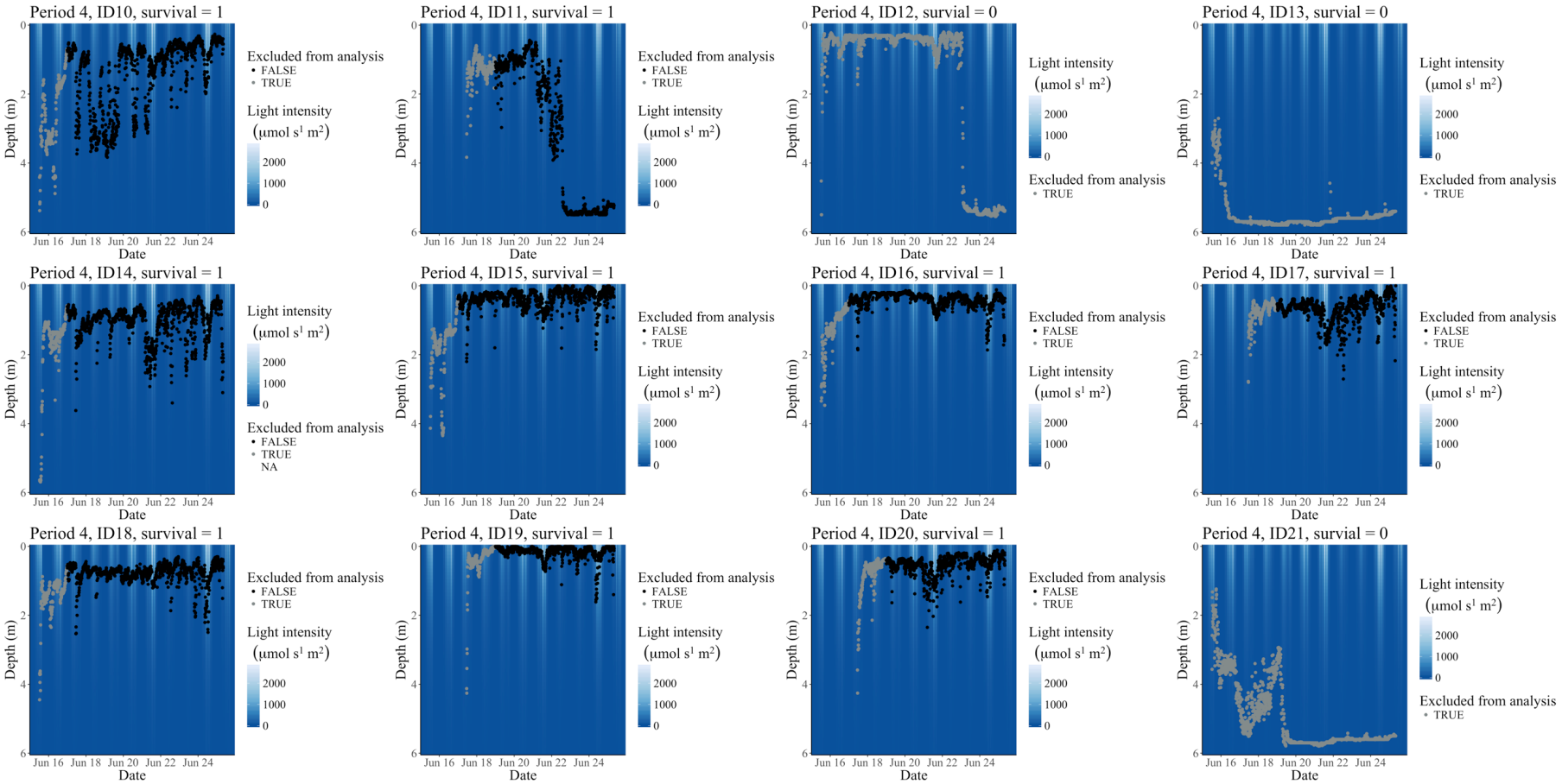


FIGURE 8A – Depth distribution of all individuals in period 4. Grey represents data excluded from the analysis either due to deviating behaviour or visiting of the study location. The base layer in the plot represents light intensity at the associated depth.

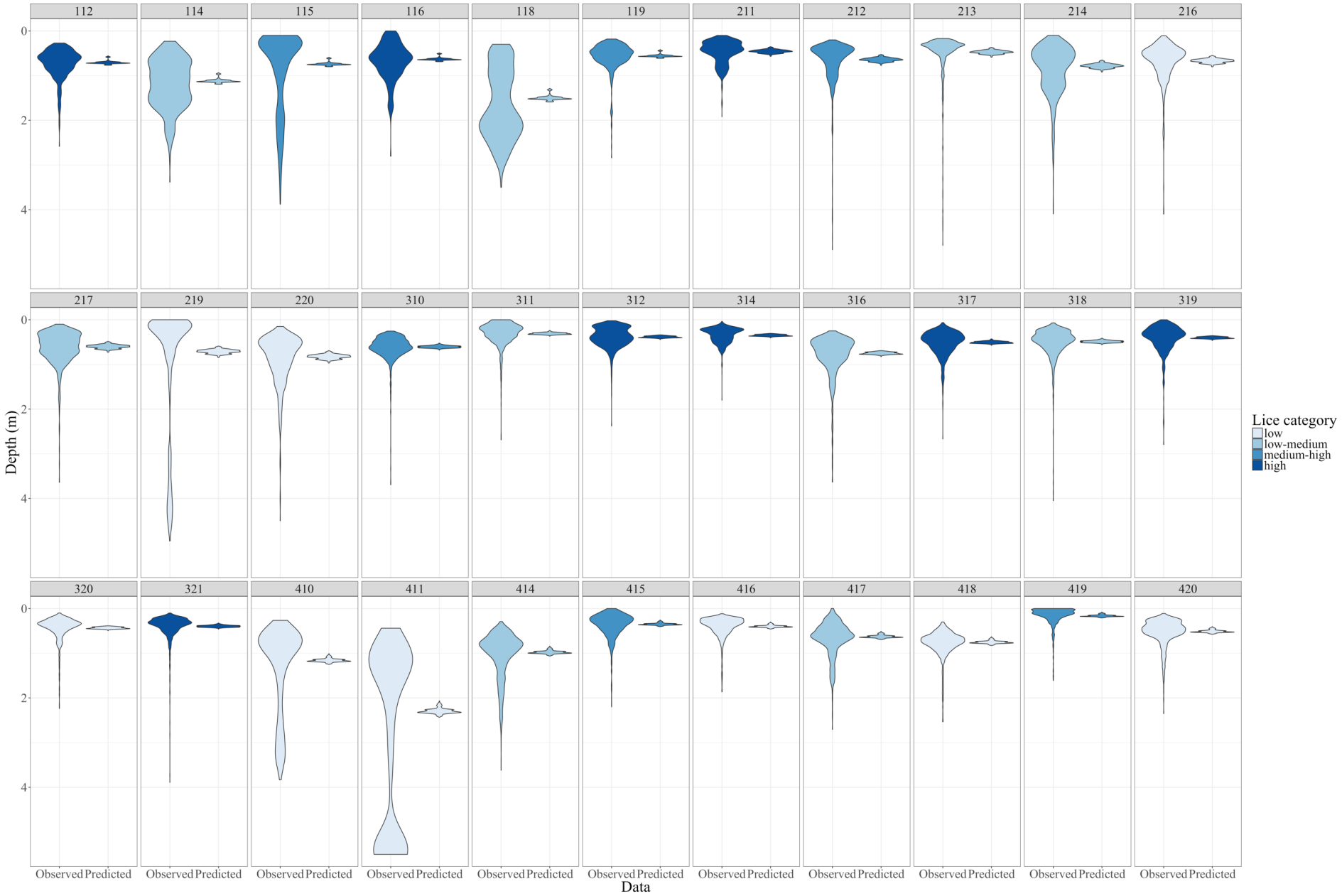


FIGURE 9A – Violin plots of observed versus predicted depth use from the top model (Table 2), retransformed from log to normal distribution, for all individuals, differentiated by category of mobile lice abundance. Categories were defined by the distribution of the data (min-Q1= low, Q1-Q2/median= low-medium, Q2/median-Q3= medium-high, Q3-max= high). First digit in the ID code represents the period of which the fish belongs to, while the two last represents the tag ID of the fish.