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The response of salmon lice nauplii and copepodids (*Lepeophtheirus salmonis*) to artificial light stimuli

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Abstract

Salmon lice (*Lepeophtheirus salmonis*) are ectoparasitic copepods that cause disease in maricultured Atlantic salmon (*Salmo salar*) and are considered one of the greatest threats to farmed and wild populations of salmonids. The parasite has become a key constraint to the continued growth of the salmonid aquaculture industry in Norway, and the annual cost of preventing and combating salmon lice infestations is high. Salmon lice larvae can detect and respond to physical and chemical cues in their external environment, and behaviour related to such cues is often associated with host-finding.

The aquaculture industry utilizes underwater lights in sea cages to delay sexual maturation and increase growth of Atlantic salmon. Light is known to trigger diurnal vertical migration in salmon lice and elicit a positive phototaxis. It is possible that current practices with artificial light in salmonid fish farms influence the concentration of salmon lice larvae inside sea cages. In this context, a field experiment where plankton samples were collected inside a sea cage, while manipulating the ambient light field with an underwater lamp, was conducted. Samples were collected during daylight hours (DH), without artificial light, and during darkness/twilight hours (DTH), with artificial light. No statistically significant difference between larval content of samples collected during DH and DTH was found ($p > 0.05$), and no conclusion regarding the effect of artificial light could be drawn.

To narrow down external factors influencing the response of salmon lice larvae, laboratory experiments were carried out on two different strains of nauplii (*Ls* Gulen and Wild) and on *Ls* Gulen copepodids. Nauplii were exposed to 3 different light stimuli (blue/green, white, violet), copepodids to 5 (blue/green, white, violet, low intensity white, high intensity violet). Control experiments, without light, were conducted for all larvae. Differences in response were found between *Ls* Gulen and *Ls* Wild nauplii: *Ls* Gulen nauplii had an increased response to blue/green and white light, while *Ls* Wild nauplii showed no significant response to any of the treatments. The contrasting response of the two nauplii strains could be due to different rearing conditions, but could also be explained by genetic dissimilarities between lice strains. *Ls* Gulen copepodids had an increased response to both violet light treatments. Use of violet light in sea cages could potentially attract copepodids to fish farms, however, absorption by particles in seawater usually impede violet light from propagating over great distances.

Sammendrag

Lakselus (*Lepeophtheirus salmonis*) er sykdomsfremkallende ektoparasitter av atlanterhavslaks (*Salmo salar*), og anses som en av de fremste truslene mot oppdrettede og ville populasjoner av laksefisk. Parasitten er en av hovedbegrensningene for vekst innen akvakultur av laksefisk i Norge, og de årlige kostnadene assosiert med bekjemping og forebygging av lakselusangrep er høye. Lakseluslarver kan oppdage og respondere på fysiske og kjemiske signaler i det eksterne miljøet, og adferd relatert til slike signaler er ofte forbundet med lokalisering av potensielle verter.

Akvakulturindustrien benytter seg av undervannslys i merder for å forsinke seksuell modning og øke vekst hos atlanterhavslaks. Lys er kjent for å utløse vertikal døgnmigrasjon hos lakselus og forårsaker en positiv fototaksis hos larvene, og det er derfor mulig at nåværende praksis med kunstig belysning i lakseoppdrett kan påvirke konsentrasjonen av lakseluslarver inne i merder. For å undersøke dette ble det gjennomført et feltforsøk, hvor planktonprøver ble samlet inn inne i en merd samtidig som omgivelseslyset ble manipulert med en undervannslampe. Prøver ble samlet inn når det var dagslys (DH), uten kunstig belysning, og når det var mørkt/tussmørkt (DTH), med kunstig belysning. Det ble ikke funnet noen statistisk signifikant forskjell av luselarveinnhold i prøvene som ble samlet inn når det var DH eller DTH ($p > 0.05$), og ingen konklusjon som gjelder effekten av kunstig lys kunne dras.

For å begrense eksterne faktorer som påvirker responsen til lakseluslarver ble et laboratorieforsøk gjennomført på nauplii av to ulike lakseluslinjer (*Ls* Gulen og Wild) og på *Ls* Gulen kopepoditter. Nauplii ble eksponert for 3 ulike lysstimuli (blå/grønt, hvitt, fiolett), kopepoditter for 5 (blå/grønt, hvitt, fiolett, lavintensitets hvit, høyintensitets fiolett). Kontrolleksperimenter, uten lys, ble gjennomført for alle larver. Det ble funnet forskjeller i lysrespons mellom *Ls* Gulen og Wild nauplii: *Ls* Gulen nauplii hadde en økt respons på blå/grønt og hvitt lys, mens *Ls* Wild nauplii ikke responderte på noen av lysbehandlingene. Kontrasterende respons som ble avdekket kan komme av ulike oppdrettsforhold, men kan også skyldes genetiske forskjeller. *Ls* Gulen kopepoditter hadde en økt respons til begge de fiolette behandlingene. Bruk av fiolett lys i merder kan derfor muligens tiltrekke kopepoditter til oppdrettsanlegg, men partikler i sjøvann absorberer vanligvis fiolett lys og hindrer at det forplanter seg over store distanser.

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Abbreviations

AOP	Apparent optical properties
BG	Blue and green
Copepodid	The third and final larval stage
CDOM	Coloured dissolved organic matter
CTD	Conductivity, temperature and depth, refers to an instrument that measures these properties
DH	Daylight hours
DTH	Darkness and twilight hours
ER	Electromagnetic radiation
FHF	Norwegian seafood research fund
FSA	Food safety authority
GLM	General linear model
HV	High intensity violet
IMR	Institute of marine research
IOP	Inherent optical properties
Larval stages	nauplius I, II and copepodid stages of salmon lice
LEDs	Light emitting diodes
LSD	Least significant difference
LW	Low intensity white
Nauplius (plural nauplii)	The first and second larval stages after hatching
NI	Nauplius I
NII	Nauplius II
NINA	Norwegian institute for nature research
NOK	Norwegian krone(r)
NTNU	Norwegian university of science and technology
NTNU SeaLab	Norwegian university of science and technology's centre of fisheries and aquaculture
PAR	Photosynthetically available radiation
R-HRS	RAMSES hyperspectral radiance sensor
S1-S4	Section 1-4 of the water column in the experimental system
Salmon lice	*Lepeophtheirus salmonis*
Sea lice	Includes several species of lice, e. g. *L. salmonis* and *Caligus elongatus*
SLRC	Sea lice research centre
TSL	Taskforce salmon lice
V	Violet
W	White

Chapter 1

Introduction

1.1 Salmon lice in Norwegian aquaculture

Salmon lice, *Lepeophtheirus salmonis*, were first described as a parasite of fish by Kröyer in 1837 (Walter, 2014). The host-parasite relationship probably dates back more than 10 000 years, and thus predates the growth of the salmon aquaculture industry (Revie et al., 2009). Salmon lice first emerged as a problem in Norway when commercial sea cage farming of salmonids was established in the 1970s (Heuch et al., 2005). Today, it is the most problematic parasite disease for maricultured Atlantic salmon, *Salmo salar*, and the aquaculture industry spends billions of Norwegian kroner (NOK) annually trying to prevent and combat salmon lice infestations (Iversen et al., 2017).

The aquaculture industry is one of Norway's most important export industries. According to Statistics Norway (2018), it produced more than of 1.3 million tonnes of salmonids in 2017 and the government wishes to increase this production five fold by 2050 (Stortinget, 2015). Salmon lice are a key constraint to the continued growth of the salmonid aquaculture industry. According to Iversen et al. (2017), direct added cost of salmon production related to sea lice amounted to 4.25 NOK per kg produced salmon, or 5 billion in total NOK in 2016. Preliminary data from 2018 indicate that the costs have further escalated (Berglihn, 2019). These estimates only cover the direct costs related to louse infestations and do not take into account the indirect costs associated with reduced growth and early harvest of infected fish.

Sea lice are considered the greatest challenge to fish health and welfare due to the severeness of infestations and damages to the fish (Hjeltnes et al., 2018). In recent years, the means of delousing fish have changed from medicinal to non-medicinal treatments due to the parasite's increased resistance to chemotherapeutants (Helgesen et al., 2018). Stress is a common denominator for fish which undergo non-medicinal treatments, causing severe strain and external damages to skin and scales. The result is often increased mortality, and as such non-medicinal delousing pose the greatest threat against the welfare of farmed fish.

In addition to the economic losses and welfare issues of the aquaculture industry, salmon lice

are considered one of the greatest threats to wild populations of salmonids (Forseth et al., 2017). The high concentration of salmon farms along the coast of Norway provides a perfect habitat for replication of salmon lice. Elevated lice biomass will affect migration of wild salmonids. As a direct result, incidents of salmon lice epizootics on wild fish stocks have increased along the coast of Norway since the 1980s, causing premature return to rivers and elevated mortality at sea (Boxaspen, 2006; Igboeli et al., 2014). Preventative measures have been taken and in 2017, the government introduced the traffic light system, a system aimed at regulating sustainable growth in the aquaculture industry (Nicholls, 2017).

1.2 Biology and ecology of salmon lice

Salmon lice are ectoparasitic copepods in the family Caligidae that feed on the skin, mucus and blood of salmonids (Igboeli et al., 2014). Infections can cause lesions and anemia in the host, which in turn can disrupt osmoregulation, lead to secondary infections and, in severe cases, be lethal (Hjeltnes et al., 2018).

1.2.1 Life cycle and development

Salmon lice have a direct life cycle and only need one host to complete the cycle from egg to fertile adult. The life cycle comprises eight developmental stages, each of which are separated by a moult (Figure 1.1) (Hamre et al., 2013). The first developmental stage, nauplii I, hatches directly into the water masses from egg strings produced by adult female lice (Samsing et al., 2016; Brooker et al., 2018). Free-living stages are planktonic and lecithotrophic, and rely on their energy reserves. Nauplii I (NI), nauplii II (NII) and copepodids comprise the free-living larval stages of the life cycle, with copepodids being the infectious stage, locating and attaching to a host. The remaining five stages of the life cycle are parasitic, and will normally be completed on a single host. (Hamre et al., 2013). Copepodids anchor themselves to the host with a frontal filament before they moult into the first of two sessile chalimus stages. Salmon lice become motile once they moult into preadults and are able to traverse the surface of the host and even change hosts. Male lice mature faster than female lice, and move across the surface of the host to locate preadult II females for precopula mate guarding (SLRC, 2019), a behaviour widely associated with crustaceans due to many species mating shortly after the female's final moulting (Elwood and Dick, 1990).

Water temperature is a key regulator of development, reproduction and dispersal of salmon lice (Ljungfeldt et al., 2017; Boxaspen, 2006). At high water temperatures, eggs develop and hatch at an increased rate, but the adult female louse produces shorter egg strings with fewer eggs. The reverse is true at cold water temperatures, but the eggs are smaller in diameter and are less viable. Temperature is especially important for the free-living stages, as they rely on their endogenous energy supply until they successfully attach to a host (Samsing et al., 2016). High water temperatures speed up the moulting process, reducing the time from egg to infec-

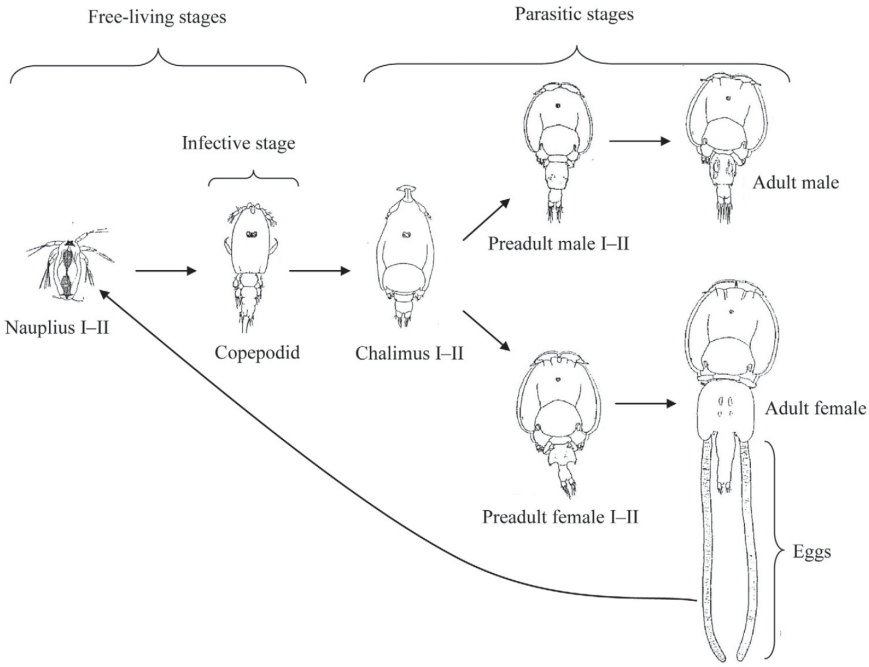

Figure 1.1: The life cycle of *Lepeophtheirus salmonis* (Igboeli et al., 2014).

tive copepodid. Elevated temperature also increases metabolic rate, causing larvae to expend their energy stores quicker, which in turn reduces larval viability. An increased developmental period increases the risk of mortality, but can also expand the dispersal distance, increasing the likelihood of an host encounter.

1.2.2 Morphology and behaviour

Salmon lice larvae must respond to both physical and chemical cues in their environment to be able to successfully locate and infect hosts (Mordue and Birkett, 2009; Brooker et al., 2018). Physical cues, such as light, pressure and salinity, probably enable the larvae to adhere to environments favoured by suitable hosts. Currents produced by swimming hosts are an example of mechanical stimuli that copepodids use for guidance to nearby hosts. In addition, odours associated with suitable hosts are known to trigger a specific swimming response associated with host-finding in copepodids.

Copepodids have several sensory structures they use in detection of physical and chemical cues, of which the antennules are their primary sensory organ (Bron et al., 1993; MacKinnon, 1993). The antennules contain both mechano- and chemosensory receptors, which suggests that they are used for detecting currents and host-associated odours in the water. Another important sensory structure is the nauplius eye, which consists of a pair of dorsal ocelli and a single ventral ocellus. The nauplius eye utilizes lens-mirror optics and has effective pigment shielding, which

is associated with enhanced directional and absolute sensitivity to light. It may also have the capacity to form images, which enables the copepodid to precisely locate a light or shadow source.

Previous studies have shown salmon lice larvae to exhibit a positive phototactic behaviour when exposed to a horizontal beam of light (Bron et al., 1993; Gravil, 1996). Gravil (1996) found that copepodids had a lower threshold for positive phototaxis than nauplii, responding to intensities as low as 3 lux (0.057 μmol photons m^{-2} s^{-1}). NI and NII first responded to intensities of 200 (3.8 μmol photons m^{-2} s^{-1}) and 85 (1.6 μmol photons m^{-2} s^{-1}) lux, respectively. A later study (Flamarique et al., 2000), where the absolute sensitivity of the visual system of salmon lice was studied, supports these findings. The authors found the absolute sensitivity of nauplii to white light to be 10^{-17} photons m^{-2} s^{-1} and the absolute sensitivity of copepodids to be 10^{-13} photons m^{-2} s^{-1}. Wavelength-specific behaviour has also been studied: Bron et al. (1993) noted that copepodids have a peak response to light of 550 nm and the lowest response to light of 400 nm; Gravil (1996) found that copepodids had the highest response to wavelengths between 500-561 nm, NII around 500 nm, and found no wavelength-specific behaviour for NI. In addition, salmon lice larvae are known to have a strong diel vertical migration (DVM) (Heuch et al., 1995), where they gather near the surface during the day and sink to deeper water masses at night. This migration pattern is thought to be an adaptation that increases host-parasite encounters, as salmonids have the reverse migration pattern of salmon lice.

1.3 Artificial light in aquaculture

The aquaculture industry utilizes underwater lights in sea cages to delay sexual maturation and increase growth of Atlantic salmon during the darkest months of the year (Oppedal et al., 1997). Light systems with metal halide lamps have long been used as a standard in salmonid aquaculture (Migaud et al., 2007). Light emitted from such lamps are perceived as bright point lights and are neither environmentally nor species specific, which could potentially compromise fish welfare. With the advance of new technology, underwater lamps with light emitting diodes (LEDs) have become more common, as they can be tuned to the environment and species sensitivities through narrow bandwidth output (Migaud et al., 2007).

Due to sea lice larvae being positively phototactic, current practices could potentially increase density of larvae inside sea cages. Hevrøy et al. (2003) found that artificial light led to an overall increase in lice infestations and that fish held at greater depths had lower infestation. Light has long been linked to vertical migration in Atlantic salmon (Huse and Holm, 1993)- In a study by Oppedal et al. (2001) it was found that Atlantic salmon swam deeper when exposed to continuous light during winter. A different study found that quantity of fish remaining deep decreased with lowered light intensity and that all light colours, except deep red, affected swimming depth (Stien et al., 2014). Light intensity and colour are known to affect phototactic behaviour of salmon lice larvae (Bron et al., 1993; Gravil, 1996; Flamarique et al., 2000; Heuch et al., 1995), thus, commercially available underwater lights known to stimulate Atlantic salmon

could potentially influence larval behaviour as well.

1.3.1 What is light?

Light is electromagnetic radiation (ER), an energy form that propagates as electrical and magnetic waves through space. ER ranges from short-waved gamma rays (10^{-14}) to radio waves (800-2000 km), and includes energy such as x-rays and visible light (Johnsen et al., 2009). Visible light ranges from around 320-760 nm and includes the spectrum of photosynthetically available radiation (PAR), which are photons with high enough radiation to drive photosynthesis ranging from 400-700 nm.

1.3.2 Optical properties of water

When measuring light one has to consider the medium it propagates through, which in the case of the experiments in this thesis is seawater. Seawater has a highly variable content of optically significant dissolved and particulate matter (Mobley, 1995). The type and concentration of solutes and particles in seawater differ, thus the optical properties show large temporal and spatial variations.

The optical properties of water can be divided into the inherent and apparent optical properties of the medium (Smith and Baker, 1981; Johnsen et al., 2013). Inherent optical properties (IOP) depend only on the medium, and remain the same regardless of changes in the ambient light field occur. IOPs specify the scattering and absorbing characteristics of the medium and its constituents (Figure 1.2), such as phytoplankton pigments, particulate detritus, and coloured dissolved organic matter (CDOM). Apparent optical properties (AOP) depend on the medium (IOPs) and the geometric structure of the ambient light field. AOPs are of particular importance when considering light penetration to depths in natural waters. IOPs and AOPs are connected by the radiative transfer equation, which is part of a larger framework that connects the optical properties of water with the ambient light field (Mobley, 1995).

The optical properties of the water column influence the distance light travels and can be measured using optical sensors (Johnsen et al., 2013). There are several types of radiance sensors with varying degrees of accuracy: RBG sensors use three wavebands centered around the red (650 nm), blue (450 nm), and green (550 nm) portions of the visible spectrum; multispectral sensors typically use 10-20 wavebands in the ultraviolet, visible and near-infrared regions of the EM spectrum; hyperspectral sensors provide hundreds of wavebands and usually detect light at 1-5 nm increments. The greater the distance between the light source and optical sensor, the greater distortion the water column has on the measured radiance.

1.4 Background for the aims of the thesis

This master thesis started out as part of a collaborative between Taskforce salmon lice and SINTEF ArmsRACE. The main objective of the research project was to study the effect of

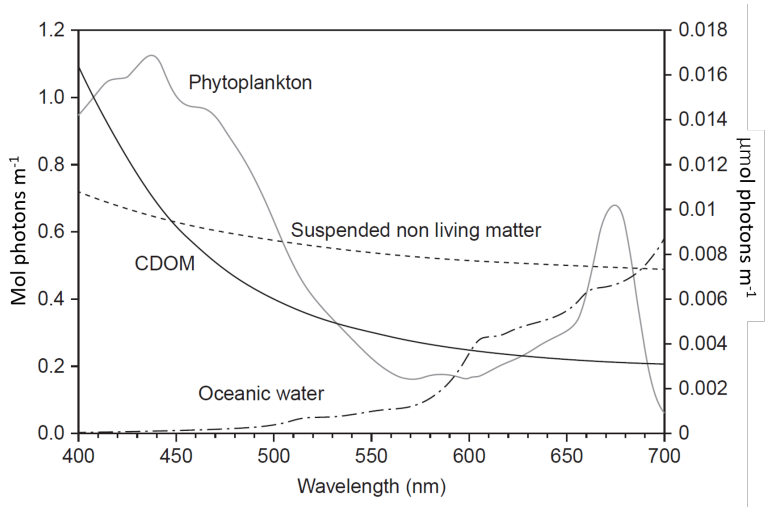

Figure 1.2: The inherent optical properties (IOPs) of the clearest oceanic seawater, Chl *c* and Chl *b*-containing phytoplankton, CDOM and suspended non living matter (Johnsen et al., 2013). All spectra use the primary Y-axis (left), except phytoplankton that uses the secondary Y-axis (right). Units: absorption, m^{-1}.

artificial lighting in sea cages on the concentration of salmon lice larvae and to assess their behaviour in relation to underwater lights used commercially today. Initially, there were two field experiments planned in cooperation with SINTEF ArmsRACE: a mesocosm experiment aimed at studying light response of salmon lice larvae in relation to specific commercial lights and a full scale experiment to directly assess the effect artificial lights have on concentration of salmon lice larvae in sea cages.

A traditional mesocosm experiment was originally planned in the early autumn of 2018; with plastic enclosures deployed in the sea, containing filtered seawater and salmon lice larvae. From this type of mesocosm, samples are usually gathered with pumps at specified locations inside the enclosure. It was determined that the resolution of such an experiment was too low and that a technological solution with high resolution cameras to track and monitor the behaviour of the larvae was desired. Planning such an experiment takes time and resources, therefore, this experiment was postponed indefinitely in December 2018. As the mesocosm experiment would not be completed within the timeline of this master thesis, the importance of the field experiment increased. The original plan was to carry out an extensive, two week experiment in the late autumn of 2018, where we'd collect samples with a plankton net inside a sea cage while manipulating the ambient light field with artificial light. The field experiment was carried out in November 2018, however, costs and effort linked to a two week field experiment were deemed too high and thus, the experimental period was reduced to three days.

In December 2018 it became clear that data collected from the field experiment alone would not be enough for a master thesis, and a new experiment had to be created. This part of the thesis was independent of SINTEF ArmsRACE, but was still based on the aims of the initial

idea. A laboratory experiment testing light response of salmon lice nauplii and copepodids was planned and designed in the spring of 2019. The idea was to build a system that resembled the hi-tech mesocosms in the original experiment. In concert with the mesocosm experiment, the laboratory experiment aimed at studying light response of salmon lice larvae in relation to specific commercial underwater lights.

1.5 Aims of thesis

This thesis aimed to investigate the response of salmon lice nauplii and copepodids to natural and artificial light, with focus on phototactic behaviour related to commercial underwater lights currently used in salmonid aquaculture production. To achieve this goal, a field experiment studying the direct effects of light on larval migration was conducted. To narrow down external factors influencing salmon lice larvae, such as natural light and water currents, a light experiment was also conducted in the laboratory. Information gained about the behaviour of salmon lice nauplii and copepodids is necessary for understanding how they disperse in the water column and locate potential hosts.

To close some of the knowledge gaps related to phototactic behaviour of salmon lice, the following research questions were raised:

1. Can current practices with artificial light in salmonid fish farms influence the abundance of salmon lice larvae inside sea cages? The following hypothesis was investigated:
    - Commercial underwater lights in sea cages can increase the concentration of salmon lice nauplii and copepodids inside the sea cage
2. Can specific wavebands associated with commercial underwater lights trigger a phototactic response in salmon lice larvae? The following hypothesis was investigated:
    - Light in the blue/green spectrum elicits a stronger phototactic response in salmon lice larvae than broad spectrum (white) and violet light does

Chapter 2

Materials and methods

Two separate experiments were carried out in this master thesis, a field experiment and a laboratory experiment, in order to investigate the questions stated in Section 1.5.

2.1 Field experiment

In November 2018, a field experiment was conducted at one of SalMar's production sites for Atlantic salmon (*Salmo salar*), Hosenøyan (Figure 2.1), which is located off the coast from Stokkøya in Åfjord municipality (Latitude: 64.087, Longitude: 9.879). This location was chosen because it contains one of SINTEF's ACE research facilities, which are sites where full-scale experiments can be carried out (SINTEF, 2019).

Plankton samples were collected inside sea cage 5 (Figure 2.1), which contained adult Atlantic salmon ready for harvest (Table 2.1). Prior to our arrival, the farm operators had counted the amount of salmon lice in the sea cage and found a total of 1 adult female and 2 motile salmon lice on 20 sampled fish.

Table 2.1: Biomass of Atlantic salmon (*S. salar*) at Hosenøyan in November 2018. Biomass in sea cage 5 (HO05) marked in bold.

Sea cage	Number of fish	Biomass (kg)	Average weight (g)
HO03	183 941	725 284	3 944
HO04	186 431	772 609	4 144
HO05	**189 192**	**830 066**	**4 387**
HO07	97 265	494 091	5 080
HO08	170 778	928 044	5 434
HO09	177 163	930 079	5 250
HO11	184 714	924 525	5 005
Total	1 189 467	5 604 698	4 712

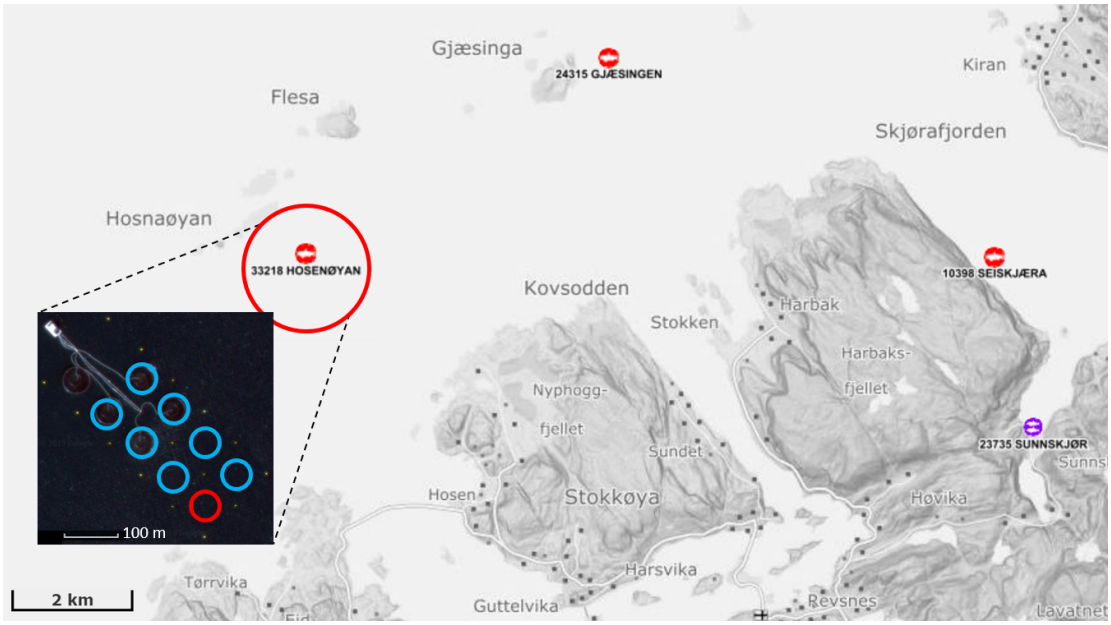

Figure 2.1: Hosenøyan, where the field experiment took place, is marked with a red circle on the map. Other aquaculture facilities in the area are also marked on the map: red dots were salmonid production sites and purple dot was salmonid hatchery (Directorate of Fisheries, 2019). Placement of sea cage 5 (red circle) in relation to other sea cages (blue circles) is shown in the recessed image (Google Maps, 2019).

2.1.1 Plankton tow sampling

Plankton samples were collected with a WP-2 plankton net (Figure 2.2) (180 cm long, Ø 570 mm, 0.25 m^2 opening; KC Denmark A/S, Silkeborg, Denmark) with a mesh size of 150 μm. A digital flow meter with back run stop (KC Denmark A/S, Silkeborg, Denmark) was mounted in the opening of the WP-2 net to register the volume of water sampled per plankton tow.

Plankton samples were collected inside the sea cage, approximately 4 meters from the net wall (Figure 2.2) as close to the artificial light as possible (∼1 m distance). The plankton net was towed with a speed of about 1 m s^{-1} from 10 meters depth to the surface, resulting in an average filtered water volume of 1.855 m^3 per tow (Appendix A). To increase the sample size, every sample included 2 plankton tows, giving a total average sample size of 3.711 m^3. The accurate water volume of each sample was calculated using Equation 2.1.

$$Nr.\ of\ revolutions\ (flow\ meter) \cdot 0.3\ m \cdot net\ opening\ area\ (m^2) = Water\ volume\ (m^3) \quad (2.1)$$

The plankton net was flushed down and the material in the cod end was filtered through a 150 μm mesh. The remaining material was transferred to 250 ml polyethylene bottles. Each plankton sample was preserved with 96% ethanol on site and diluted down with seawater to a

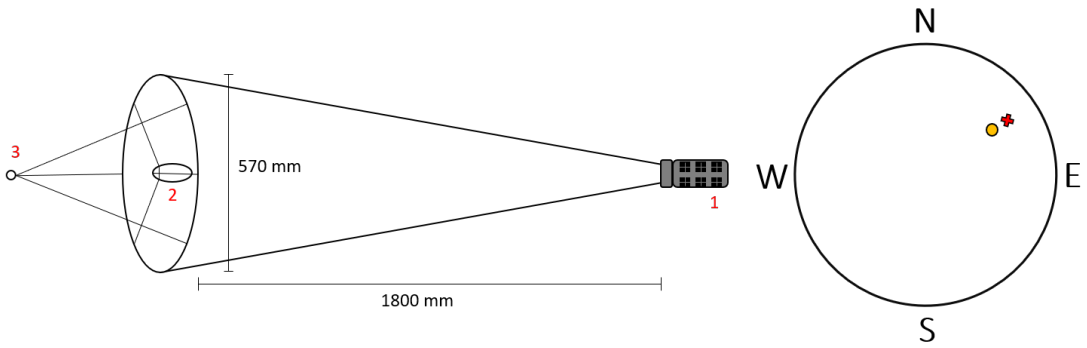

Figure 2.2: Left: Schematic representation of WP-2 plankton net used for planton tow sampling during field experiment: 1) cod end, 2) digital flow meter, 3) attachment mechanism. **Right** Schematic representation of sea cage 5. Position of artificial light and plankton tow sampling site are marked with a yellow circle and a red X, respectively. Sea cage not drawn to scale.

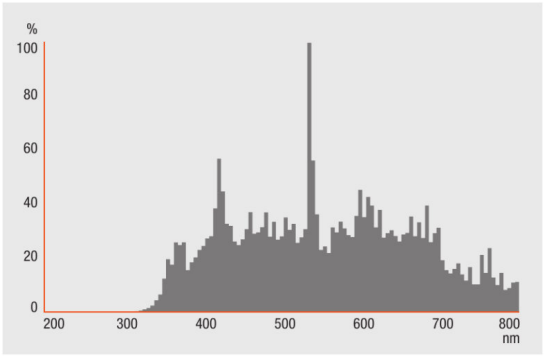

Figure 2.3: Relative power distribution of the artificial light source used in the field experiment (Osram, 2019).

concentration of around 70% ethanol. In total, 40 plankton samples were collected during the field experiment.

Light conditions

An artificial light source (Figure 2.3) was located at approximately 1 m distance from the sampling site at 5 m depth. The ambient light field was manipulated with an underwater lamp. 20 samples were collected with the artificial light turned off during daylight hours (DH), and 20 samples were collected with the artificial light turned on during darkness/twilight hours (DTH) (Table 2.2) (Appendix B).

Table 2.2: Light conditions during plankton tow sampling at Hosenøyan on Nov 6-7th during daylight hours (DH) and darkness/twilight hours (DTH) (Appendix B).

Time of day	Natural light	Artificial light	# of samples
DH	+	-	20
DTH	-	+	20

2.1.2 Abiotic measurements

Diffuse light was measured with a RAMSES hyperspectral radiance sensor (R-HRS) (TriOS, Rastede, Germany) at 0, 1, 2, 4, 6, 8, and 10 m depth outside the sea cage. These measurements were done at 13:00 on Nov 6th (sunset at 15:47), while the sun was shining and the cloud cover was limited. Light intensity at the surface was not measured simultaneously with the diffuse light measurements, thus, shifts in intensity (e.g. due to cloud cover) could not be accounted for. Temperature and salinity profiles were measured using a CTD (SD 204; SAIV A/S, Bergen, Norway) both inside (0-10 m) and outside (0-25 m) sea cage 5.

Measurements of water currents inside sea cage 5 were provided by SKJERMTEK (FHF project number 901396 (FHF, 2017)). The currents inside sea cage 5 were registered by four single-point current meters (Nortek Vectors, Rud, Norway), placed as close to the sampling site as possible during most of the plankton tow sampling. The current meters were mounted in a vertical line at 2, 4, 6, and 8 m depth, registering direction and velocity of horizontal currents every second.

2.1.3 Sample analyses

Collected samples were stored in cooling boxes after fixation with ethanol. Three of the samples (# 3, 4, and 6) were transported by boat and analysed visually at the feed barge, the remaining samples were transported by boat to the mainland and by car to the Norwegian University of Science and Technology's Centre of Fisheries and Aquaculture (NTNU SeaLab). Here, the samples were stores in a cooling room (4 °C) until analyses could be conducted. Two of these samples (# 34 and 40) were analysed visually at NTNU SeaLab, the remaining 35 samples were sent to the Norwegian Institute for Nature Research (NINA) for genetic analysis with Digital Droplet™ Polymerase Chain Reaction (ddPCR). None of the samples were analysed with both methods.

Visual analysis of plankton samples

Plankton samples were filtered through a 120 μm mesh to remove ethanol, and diluted with filtered seawater into glass beakers. Subsamples ($<$ 1 mL) were extracted and placed into 4-well counting chambers ($<$ 0.25 mL per well). Counting chambers were then placed under a stereo microscope (Leica M80; Leica Microsystems, Wetzlar, Germany) and particles in subsam-

ples were visually analysed (1.6-2.0 magnification) to determine if they were salmon lice. This procedure was repeated until all particles of plankton samples had been visually analysed.

DNA isolation and detection of genetic markers with ddPCR

DNA extraction and ddPCR analysis took place in laboratory facilities designed for handling environmental samples at NINA, Trondheim. Plankton material of samples was crushed and homogenized before three subsamples were extracted from each sample. These subsamples were dried in a heat cabinet to remove excess EtOH before the protocol DNeasy Blood & Tissue kit (Qiagen, Carlsbad, CA, USA) was used for DNA extraction (Spens et al., 2017).

Following DNA extraction, PCR droplets were generated in an AutoDG^{TM} Instrument (Bio-rad Laboratories, Inc., hercules, California, USA) and PCR amplification was performed in a Veriti^{TM} 96-Well Thermal Cycler (Applied Biosystems, Foster By, California, USA). PCR plates were then transferred to a QX200^{TM} Droplet Reader (Bio-rad Laboratories, Inc.) for automatic detection of genetic markers in droplets. The manufacturer's software (QuantaSoft, Bio-rad) was used to separate positive from negative droplets (Wacker et al., 2019).

2.1.4 Statistical analyses

To determine what model would best fit the data, Shapiro-Wilk tests of normality and a plot of residuals were done in IBM SPSS Statistics (Appendix C).

To determine whether light conditions had an effect on larval content of plankton samples, a general linear model (GLM), with a confidence interval of 95%, was used to test variance between samples collected during DH and DTH in IBM SPSS Statistics.

2.2 Laboratory experiments

2.2.1 Source of salmon lice and culture maintenance

Taskforce Salmon Lice (TSL) maintained several cultures of salmon lice at NTNU SeaLab. Including an *L. salmonis* strain (*Ls* Gulen) reared for more than 70 generations at the Sea Lice Research Centre (SLRC) (Hamre et al., 2009) and a strain of *L. salmonis* (*Ls* Wild) collected from wild salmon captured at Agdenes.

TSL's salmon lice cultures were reared on Atlantic salmon in eight 400 L tanks (100 cm length x 100 cm width x 50 cm height) in a climate controlled room with a stable temperature of 10.0 °C. Temperature in the tanks varied with season, but remained stable between 7.6 - 10.6 °C. Salinity ranged from 23-33, water flow was between 450-500 L h^{-1}, and fish were exposed to continuous light (16:8 light:dark for *Ls* Gulen experiments). There were 8-12 fish in each tank (from 2-600 g, ± 50 g within each tank). Salmon lice load varied between fish, however, a maximum limit of 10 female lice + 10 male lice per fish was set by the Norwegian Food Safety Authority (FSA), which was monitored by responsible personnel.

Infection protocol

Salmon lice used for infection were reared in the hatchery until they reached copepodid stage. Copepodids were then extracted from incubators and 4-600 copepodids (50 per fish in tank) were collected and transported in glass beakers to the culture room. Water flow to the tank, with fish that were infected, was stopped and water level reduced by $\frac{1}{3}$. Copepodids in the glass beaker were then spread evenly into the tank. The flow remained off for 30 minutes - to allow copepodids to locate and attach to the fish - before it was set to 250 L h^{-1} for an additional 30 minutes. After a total of 60 minutes, the water flow was returned to normal (450-500 L h^{-1}) and infection was completed.

2.2.2 Harvesting of *Ls* Gulen and *Ls* Wild

Salmon lice used in this study were harvested from TSL's cultures (both *Ls* Gulen and *Ls* Wild). When harvesting egg strings, the fish were first sedated in the tanks with 15-20 mL Benzoak (benzocaine as active agent, $\frac{1}{4}$ of standard dose) (5 min). Fish carrying female sea lice, with egg strings ready for harvest, were transferred, one at a time, into a container holding 5 L seawater, 25 μg Aquacalm™ (metomidate hydrochloride), and 1,5 ml Benzoak. Once the fish was properly sedated (5 min), it was transferred into a shallow tray with seawater and the egg strings were harvested from the lice with tweezers and placed into beakers containing seawater. The maximum handling time of the fish, from start of sedation until return to tank, was 15 minutes. When the harvest was completed, the fish were returned to their tank and egg strings were transferred to incubators in the hatchery, with a maximum of two egg strings per incubator well.

The hatchery was kept in a climate controlled room, with a stable temperature of 10.0 °C and a diurnal light cycle of 16:8 (light:dark). Temperature of the seawater was 9.0 ± 0.2 °C and salinity was between 32-33. Incubators in the hatchery were crafted after an incubator recipe published by the Institute of Marine Research (IMR) (Hamre, 2013) (Figure 2.4). It roomed 16 incubator wells per incubator, each with a volume of 38 ml and a water flow of 38 ml min^{-1}. For the experiments with *Ls* Wild nauplii, the incubators were modified and the flow was reduced to 3 ml min^{-1}, controlled by a tubing pump (Watson Marlow 205-U, Falmouth, UK). Intake water at NTNU SeaLab was pumped from approximately 70 m depth and particles <70 μm were removed with a sand filter. In addition, the hatchery's water supply was filtered through two 10 μm filters, however, barnacle larvae were observed in incubator wells.

2.2.3 Experimental setup

Experiments took place in a climate controlled room with a system designed and produced in cooperation with NTNU SeaLab technicians (Figure 2.5). To mimic the natural environment of salmon lice, a vertical system was chosen to include the effect of gravity. The system consisted of a dark grey PVC pipe (Ø 50 mm) with a roughened interior surface to reduce light reflections

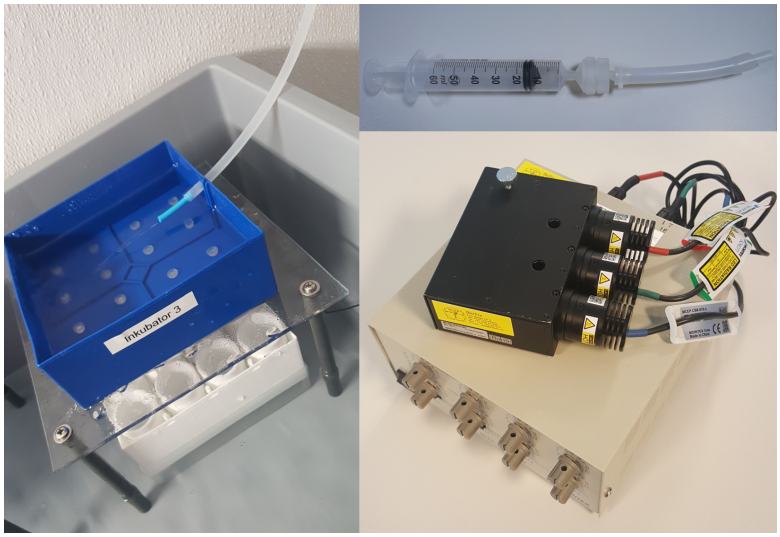

Figure 2.4: Left: Incubator in *L. salmonis* hatchery at NTNU SeaLab. **Top right:** Modified syringe used to insert salmon lice larvae into the experimental system through a valve. **Bottom right:** LED light system used in the laboratory experiments.

in the material. It had a total length of 185 cm, sectioned into three 50 cm pieces and one 35 cm piece (top section). A valve for draining seawater was placed at the base of each section, and the sections were joined together by muffs above the three top valves. A draining valve was also installed at the bottom of the system, used for emptying the sedimentation chamber. Total length of the water column inside the pipe was 175 cm, regulated by a hole drilled into the top of the pipe. Artificial light was supplied through a separate system (detailed in Subsection 2.2.3), which connected to the collimator attached to the top of the system.

Artificial light system specifications

The light system consisted of two 3-channel LED controllers (MLEP-A070W3LR; MORITEX Corp., Saitama, Japan) mounted with LED spotlights (MCEP-Cx-070; MORITEX Corp., Saitama, Japan) which were fitted with 3W 700mA lambertian LEDs: violet (peak 405), blue (peak 450), green (peak 535) and white (peak 600) (Figure 2.4).

Two 3-channel LED mixers (MCEP-AD3LGC; MORITEX Corp., Saitama, Japan), fitted with the LED spotlights, were fed into a bifurcated fiber light guide (MWG7-1000S; MORITEX Corp., Saitama, Japan). To overcome the lack of randomization in the fiber bundles of the bifurcated light guide, the other end of the light guide was spliced onto the end of a liquid-filled light guide (Ø 5mm, Lumen Dynamics/Excelitas Technologies Corp., Waltham, MA, US). This was then fed into the experimental system through a collimator (C-HGFIB; Nikon Corp., Tokyo, Japan) modified to accommodate the delivering end of the liquid light guide.

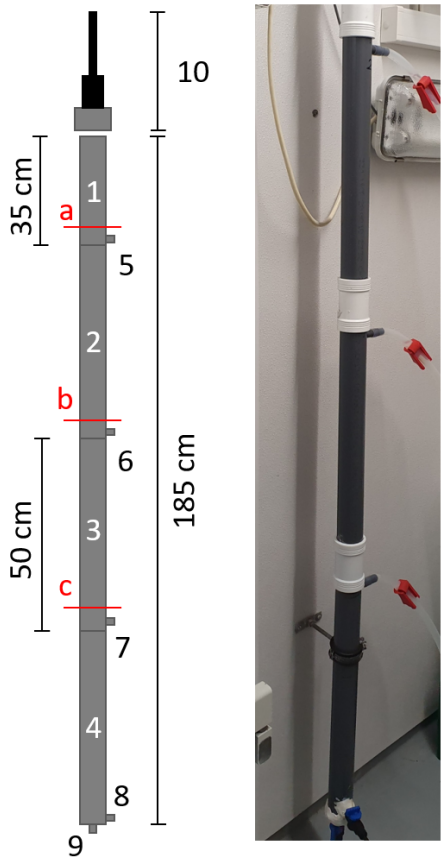

Figure 2.5: Left: Schematic representation of experimental system used in laboratory experiments: 1-4) different sections of PVC pipe (1 at top), 5-8) draining valves installed at the base of each section, 9) draining valve installed at the bottom, 10) collimator, a-c) muff connections. **Right:** The system light experiments on salmon lice larvae were conducted with.

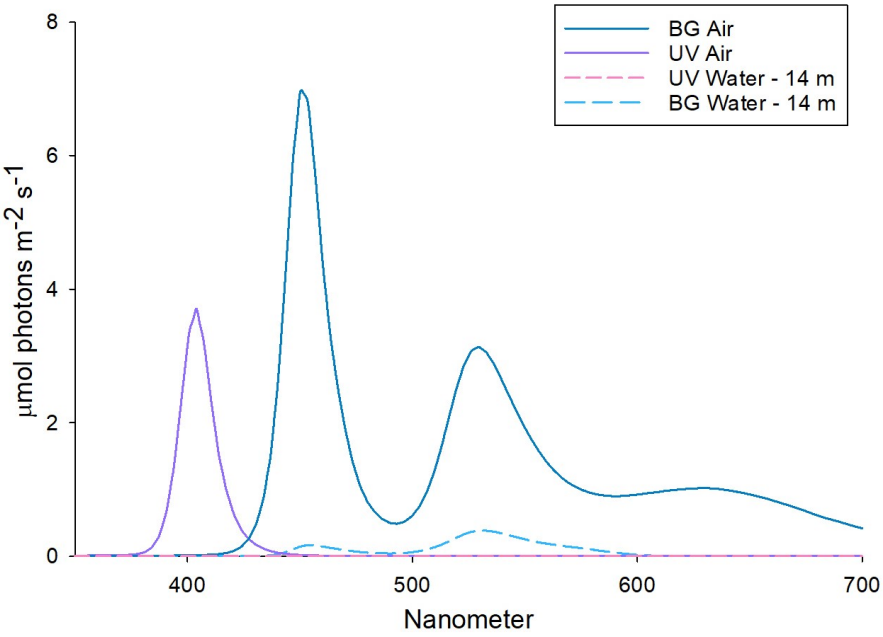

Figure 2.6: Spectral distribution of underwater lamp used as reference for laboratory experiments (BG + UV Air). Calculated light attenuation of the lamp at 14 m distance from the lamp (BG + UV Water - 14 m), in seawater relevant to fish farms, added as a reference.

2.2.4 Modeling of artificial light

Intensity and colour of artificial lights used in experiments were chosen based on current practices with artificial light in salmonid fish farms, with focus on new technology. For this reason, LEDs were used for light experiments with salmon lice larvae in the laboratory.

To relate experiments to field conditions, LEDs fitted in the light system (Section 2.2.3) simulated the spectral composition of an underwater lamp commercially used in aquaculture today, with blue, green, white and ultraviolet LEDs (Figure 2.6). Spectral composition of the lamp was measured in a dark room in air with a R-HRS. Due to risk of overheating, measurements were not done when the lamp was at maximum capacity.

LEDs used in experiments could not replicate the full intensity of the lamp and at such intensities, study animals would have been overwhelmed (Figure 2.7). To get an understanding of what distance the simulated spectrum represented to the lamp underwater, diffuse light measurements from the field experiment were used to calculate the attenuation of light emitted by the lamp (Section 2.1.2).

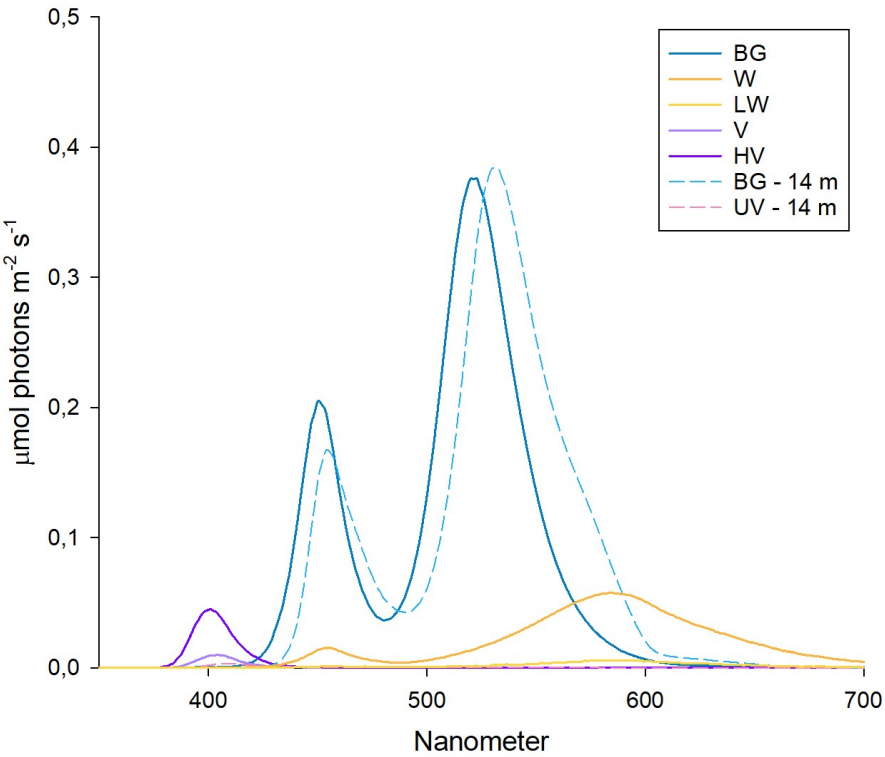

Figure 2.7: Spectral distribution of light treatments used in laboratory experiments: blue/green (BG), white (W), low intensity white (W), violet (V), and high intensity violet (HV). Calculated light intensity of underwater lamp at 14 m distance from the lamp (BG + UV - 14 m), in seawater relevant to fish farms, added as a reference.

Calculation of light attenuation

To determine light attenuation of the underwater lamp in water masses relevant to fish farms, the diffuse attenuation coefficient (K_d) was calculated from measured data collected at Hosenøyan (Equation 2.2, where E_d is measured irradiance and z is depth).

$$K_d(z_1 \leftrightarrow z_2) = \frac{1}{z_2 - z_1} \cdot \ln \frac{E_d(z_1)}{E_d(z_2)} \tag{2.2}$$

$$\overline{x} = \frac{x_1 + x_2 + ... + x_n}{n} \tag{2.3}$$

Average spectral K_d (1 nm resolution) was calculated (Equation 2.3) and used to determine irradiance from the lamp with increasing distance (Equation 2.4, where E is irradiance, λ is wavelength, z is distance from light source, s is light source, and k is K_d(lambda)).

$$E_z(\lambda) = E_s \cdot e^{-kz} \tag{2.4}$$

Output of LEDs, fitted in the light system (Section 2.2.3), was measured with a R-HRS at the top and bottom of the experimental system to determine the amount of light lost due to scattering of light and the restrictive nature of the PVC pipe (Section 2.2.3). For practical reasons, these measurements were done without water in the system.

2.2.5 Experimental procedure

Prior to the main experiments on salmon lice larvae, several pilot studies aimed at improving system design and experimental methodology were conducted. This included (but was not limited to): a test of different types of pipes (transparent and opaque); an examination of light reflections in materials (and how to reduce them); an improvement of the injection mechanism (to reduce loss of animals due to larvae adhering to surfaces); a study of larval dispersion in the system due to injection mechanism; preliminary experiments to discover when majority of nauplii and copepodids reach the top of the water column (to determine duration of experiments); a test of the effect acclimatization to darkness had on the larvae. The culmination of these tests resulted in the experimental procedure that follows.

Separate experiments were carried out on *Ls* Gulen nauplii II, *Ls* Wild nauplii II, and copepodids. Salmon lice moulted to nauplii II approximately 27 hours post hatching and reached copepodid stage around 120 hours post hatching (9.0 ± 0.2 °C) (Eichner et al., 2014). Nauplii II were used in experiments between 48-96 hours post hatching, copepodids were used in experiments within 7 days post moulting.

Experiments with four different light treatments were conducted on nauplii (both strains), six on copepodids (Table 2.3). Each experiment consisted of three replicates, and 110 nauplii or copepodids were used per replicate. *Ls* Wild nauplii and copepodids were acclimatized to darkness (30 and 60 min, respectively) prior to the start of each experiment, and were not

Table 2.3: Light colours and intensities used in laboratory experiments, and lice strains and stages exposed to each treatment (n = 3): D) dark, BG) blue/green, W) white, V) violet, LW) low intensity white, HV) high intensity violet, N) nauplii, C) copepodids.

Light colour	Light intensity (μmol photons m^{-2} s^{-1})	*Ls* Gulen nauplii	*Ls* Wild nauplii	*Ls* Gulen copepodids
D	-	x	x	x
BG	23	x	x	x
W	6.1	x	x	x
V	0.24	x	x	x
LW	0.58			x
HV	1.1			x

exposed to any light before they entered the experimental system. Experiments conducted on *Ls* Gulen nauplii were completed before acclimatization to darkness was tested and included in the experimental methodology.

Experiments on *Ls* Gulen nauplii were part of preliminary experiments to determine experiment duration. Due to complications with the hatchery and time constraints, results from these experiments will be presented in the main findings (Chapter 3). For this reason, experiment duration varied between larval groups; experiments on *Ls* Gulen nauplii had either 3, 5 or 10 min duration, while experiments on *Ls* Wild nauplii were 7 min. Experiment duration for *Ls* Wild nauplii was chosen based on preliminary experiments with *Ls* Gulen nauplii, acclimatized to darkness, which revealed that they displayed a similar phototactic response as nauplii not acclimatized, albeit with a slower response time. Experiment duration for copepodids was either 7 or 10 min. Copepodids in preliminary experiments had not been acclimatized to darkness and an assumption was made that their response time would increase with acclimatization, as it did for nauplii. Thus, the experimental duration was increased (from 5 and 7 min) for the main experiments.

Ls Wild nauplii or copepodids were collected from incubators and 110 larvae were counted and placed into a 10 mL silicone tube, connected to a modified syringe (Luer lock 50 ml; BD Plastipak, Franklin Lakes, New Jersey, USA) filled with 20 mL seawater from the hatchery (Figure 2.4). A 120 μm filter was inserted into a filter holder (Swinnex 25 mm; Merck Millipore, Burlington, Massachusetts, USA) between the tube and syringe. The syringe containing larvae, either nauplii or copepodids, was then stored in a Styrofoam box for either 30 or 60 minutes (nauplii or copepodids, respectively).

Seawater used to fill the experimental system was collected from the hatchery's water supply and stored in plastic containers in the room where experiments took place. The experimental system was filled with seawater 15 or 30 min (*Ls* Wild nauplii or *Ls* Gulen nauplii and copepodids, respectively) before the start of each experiment to allow seawater to settle in the pipe. Once the water was settled and/or the acclimatization period was completed, larvae were injected into the experimental system through a valve at the base of section 4. After injection,

light stimulus was switched on and the experiment ran its duration.

Upon completion, larvae were drained from valves at the base of each section, from top to bottom, and placed into separate plastic containers. Seawater and larvae from each section were then filtered through a 120 μm mesh, to remove excess seawater, and larvae were stored in glass beakers for further visual analyses with a stereo microscope (Section 2.1.3). Each section of the system was disconnected and flushed, to include larvae adhering to walls in results. Larvae remaining in the syringe and that had sedimented to the bottom were also stored in glass beakers and visually analysed, but not included in results.

2.2.6 Statistical analyses

To determine what model would best fit the data, Shapiro-Wilk tests of normality and a plot of residuals were done in IBM SPSS Statistics (Appendix C).

To discover if experiment duration had an effect on larval travel distance in the system (*Ls* Gulen nauplii and copepodids), analyses with GLMs were done in IBM SPSS Statistics. GLMs were also used to compare how different light treatments affected travel distance of larvae in the system (both nauplii strains and copepodids). These analyses compared variation in travel distance of larvae between all light treatments and in all sections of the system. If significant variation in larval travel distance due to light treatment was discovered, additional analyses with GLMs and post hoc LSD tests were done for each section to clarify this variation. All analyses had a confidence interval of 95%.

For the two nauplii strains, differences in light response were analysed with two tailed t-tests (significance level $p < 0.05$), comparing each treatment within each section for *Ls* Gulen to corresponding treatment and section for *Ls* Wild.

Chapter 3

Results

3.1 Field experiment

3.1.1 Abiotic conditions

Temperature and salinity profiles down to 10 m depth inside sea cage 5 (Figures 3.1 and 3.2) remained stable during the sampling period on Nov 6-7th. Temperature ranged from 8.8 to 8.9 °C and salinity from 33.7 to 33.9. Salinity and temperature profiles down to 25 m depth outside sea cage 5 revealed a thermocline at approximately 16 and 13 m depth (on Nov 6th and 7th, respectively), with temperatures from 8.6 to 9.5 °C and salinity from 32.4 to 35.1. Both temperature and salinity increased with depth.

Water currents, measured by four single point current meters at 2, 4, 6, and 8 m depth inside sea cage 5, mainly moved in an E-NE direction (Figures 3.3 and 3.4) with a mean horizontal current velocity between 0.06-0.18 m s^{-1} (Figure 3.5). Registered maximum velocities varied between 0.20-0.80 m s^{-1}.

3.1.2 Plankton samples

Water volume and larval content varied between plankton samples (Appendix B), however, average water volume was 3.711 m^3 and mean larval content was 0.3 larvae m^{-3}. Majority of salmon lice larvae were found in plankton samples collected during DH (Figure 3.6) between 08:16-15:47, with an average of 0.4 larvae m^{-3}. In comparison, an average of 0.2 larvae m^{-3} were found in plankton samples collected during DTH (07:21-08:16, 15:47-16:42). A GLM analysis revealed that there was no significant variance ($p > 0.05$) in larval content between samples collected during DH and DTH, which means that differences in larval concentration could be chance findings and not a direct effect of light conditions.

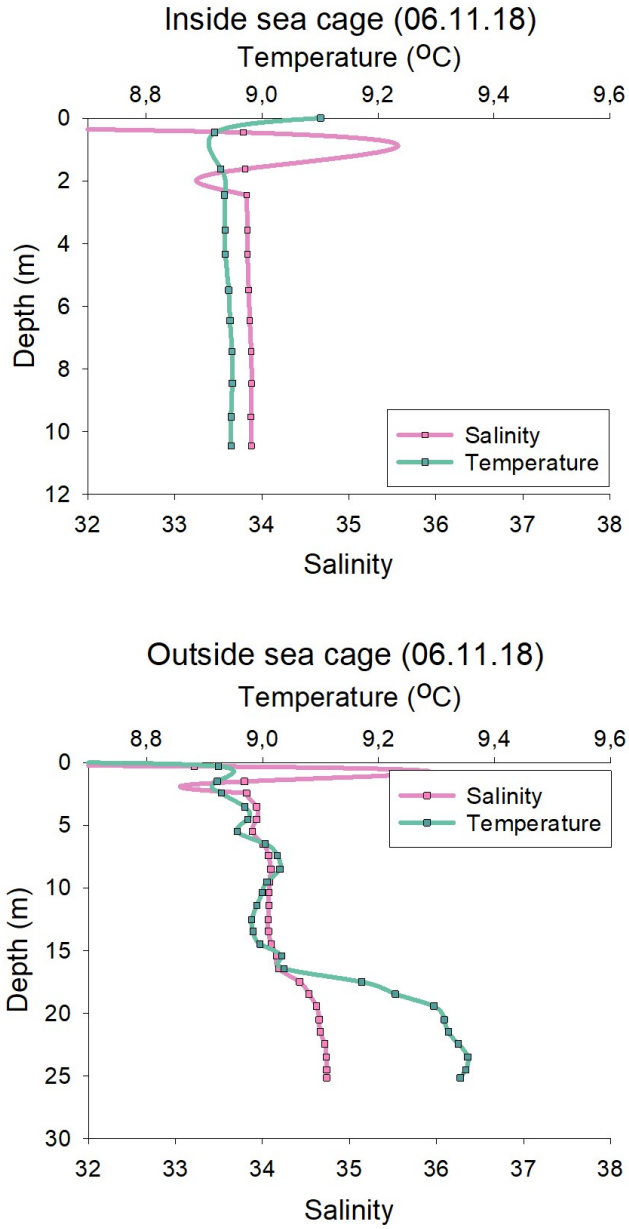

Figure 3.1: Salinity and temperature profiles inside (0-10 m) and outside (0-25 m) sea cage 5 at Hosenøyan on Nov 6^{th}.

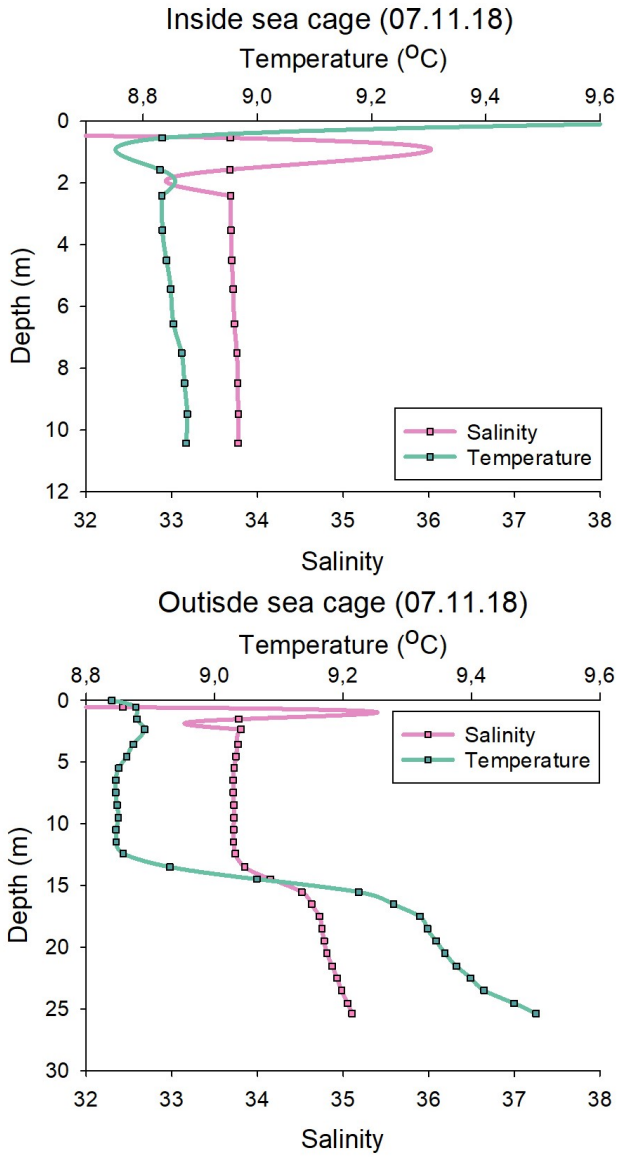

Figure 3.2: Salinity and temperature profiles inside (0-10 m) and outside (0-25 m) sea cage 5 at Hosenøyan on Nov 7^{th}.

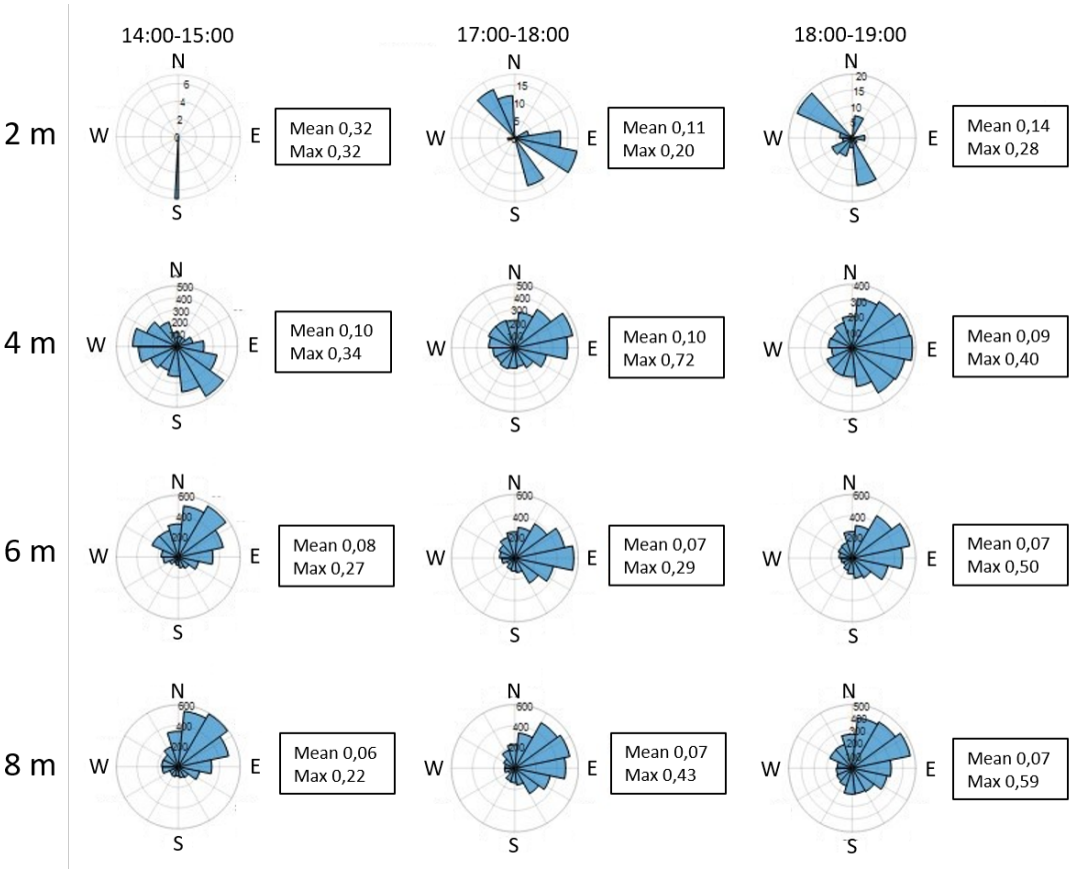

Figure 3.3: Current direction at 2, 4, 6, and 8 m depth between 14:00-15:00 and 17:00-19:00 on Nov 6^{th}. Mean and maximum horizontal current velocity (m s^{-1}) has been noted next to each plot. The top current meter (2 m) did not function properly and has been disregarded in results.

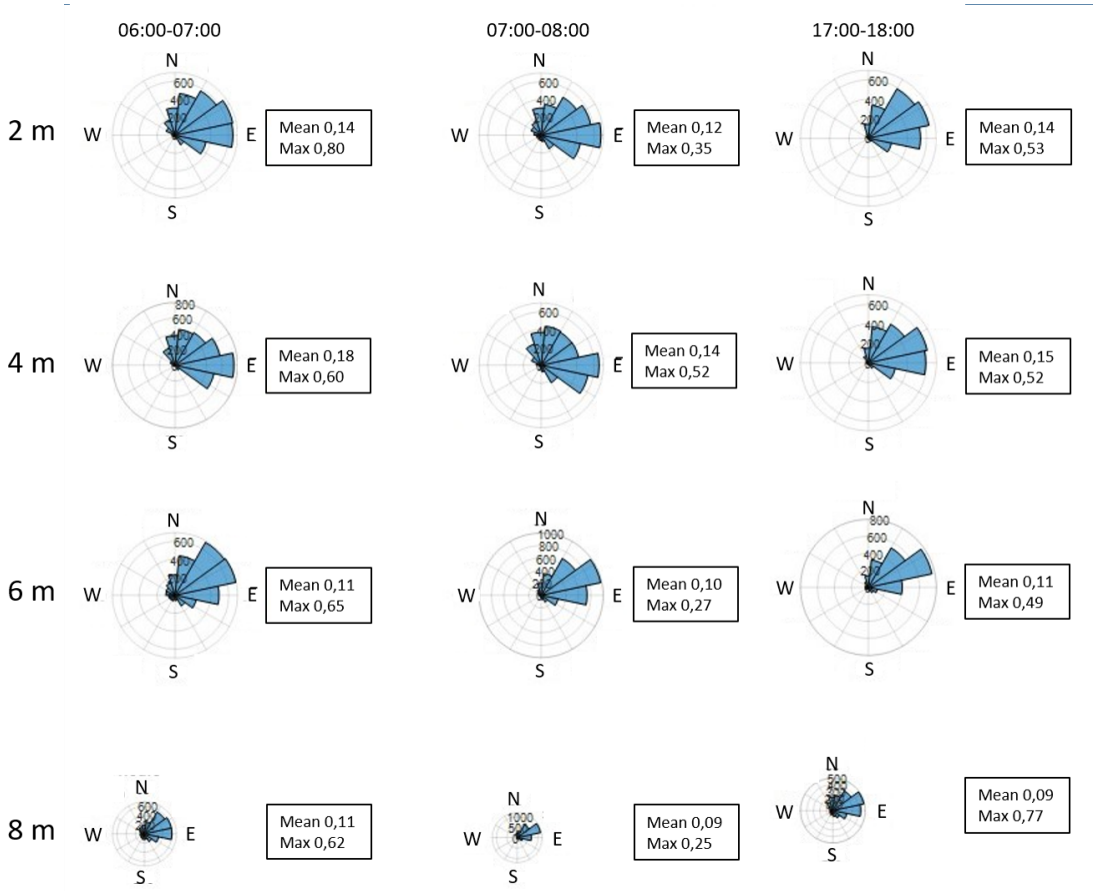

Figure 3.4: Current direction at 2, 4, 6, and 8 m depth between 06:00-08:00 and 17:00-18:00 on Nov 7^{th}. Mean and maximum horizontal current velocity (m s^{-1}) has been noted next to each plot.

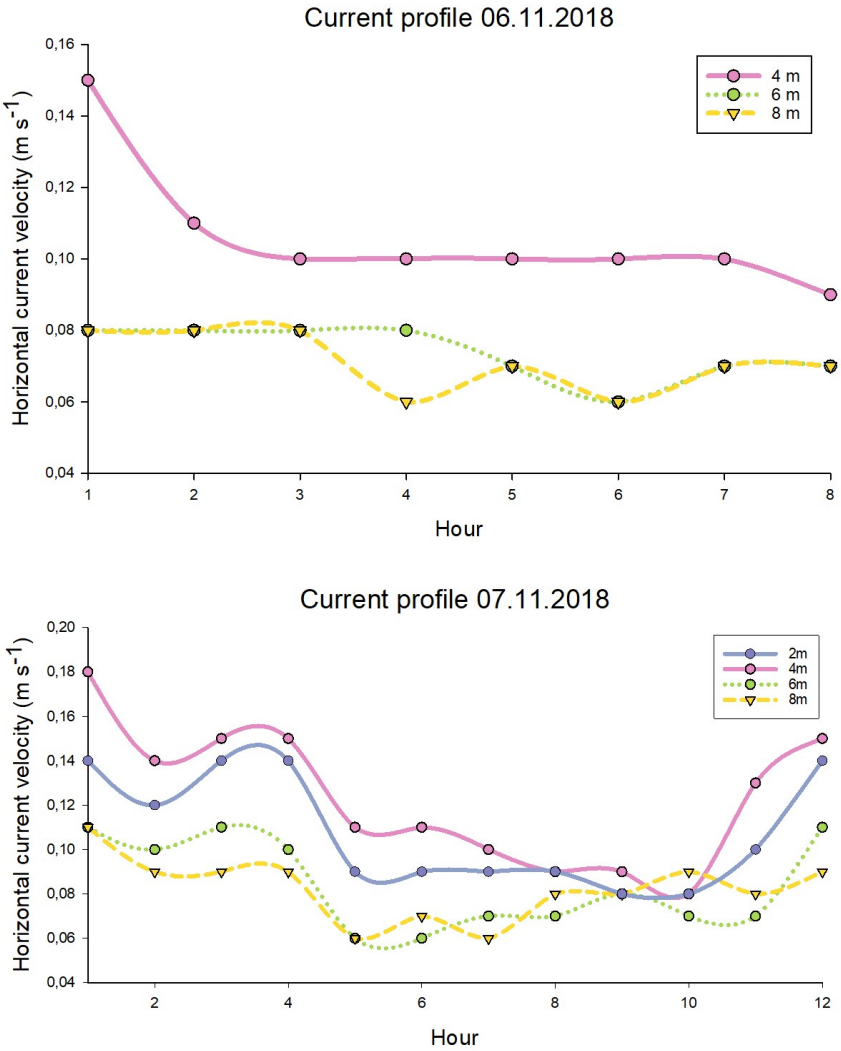

Figure 3.5: Hourly registration of mean horizontal current velocity (m s^{-1}) between 11:07-19:20 on Nov 6^{th} and 05:55-19:00 on Now 7^{th}, at 2 (only on Nov 7^{th}), 4, 6, and 8 m depth.

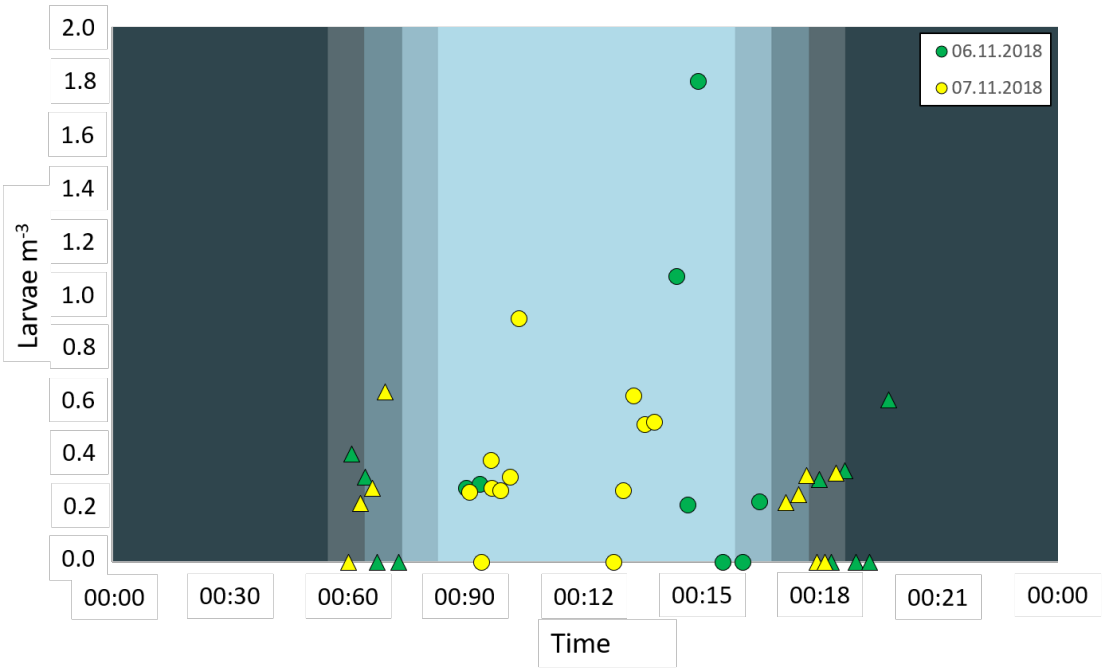

Figure 3.6: Salmon lice larvae m^{-3} vs. sampling time on Nov 6-7th 2018. Samples collected during DH without artificial light are marked with circles, while samples collected during DTH with artificial light are marked with triangles. The background is a graphic representation of natural light on Nov 6th, with sunrise at 08:16 (dawn start 07:21) and sunset at 15:47 (dusk end 16:42).

3.2 Laboratory experiment

LED output at the top of the water column corresponded to a modeled distance of approximately 11 m to the underwater lamp. Light measurements at the bottom of the pipe revealed that scattering of light and the restrictive nature of the PVC pipe further decreased LED output and increased distance to the lamp by 3-4 m. Nauplii and copepodids were therefore experiencing light intensities corresponding to a distance of 14-15 m to the underwater lamp when inserted into the bottom of the experimental system.

Shapiro-Wilk tests found that data was not normally distributed ($p < 0.001$), but had a Poisson distribution (Appendix C). For this reason, univariate GLMs were determined as the best fit for data.

3.2.1 Experiment duration

GLMs analysing whether experiment duration, for experiments with *Ls* Gulen nauplii or copepodids, had an effect on travel distance of larvae revealed no significant variation between replicates due to time ($p > 0.05$ for both stages). To increase the power of further statistical analyses, experiments with *Ls* Gulen nauplii (3, 5, and 10 min duration) were pooled into one group (Figure 3.7), copepodid experiments (7 and 10 min duration) were also pooled (Figures 3.8 and 3.9).

3.2.2 Effect of light treatment on travel distance

Ls Gulen nauplii and copepodids were evenly distributed between the four sections of the water column for treatment D (control) (Figures 3.10 and 3.11). An upward migration towards the light source was apparent for all light wavebands (including D), however, a GLM analysis of the effect of light treatment on travel distance revealed no significant variation between different treatments ($p > 0.05$). Nevertheless, there is a strong and significant interaction between light waveband and travel distance ($p < 0{,}001$). This shows a general effect of light (compared to D) eliciting a positive phototactic response in *Ls* Gulen nauplii (S1: $p < 0.05$, S2: $p > 0.05$, S3: $p > 0.05$) and copepodids (S1: $p > 0.05$, S2: $p > 0.05$, S3: $p > 0.05$), reducing the density of copepodids in the lower part of the water column (S4: $p > 0.05$ and $p < 0.002$, respectively). The interaction indicates that some light wavebands have a stronger effect on positive phototactic behaviour than others. Attempts to resolve this interaction by performing an LSD post hoc test for each section remained largely unsolved. However, there was a clear tendency that blue/green and white light caused the greatest response in *Ls* Gulen nauplii and that violet light elicited the strongest response in copepodids.

In general, majority of *Ls* Wild nauplii were not actively moving in the water column regardless of treatment ($p > 0.05$) and most remained stationary within S4 throughout the experiment. Lack of interactions between light waveband and travel distance ($p > 0{,}05$) also clearly shows that no light colour influenced migration (Figure 3.10).

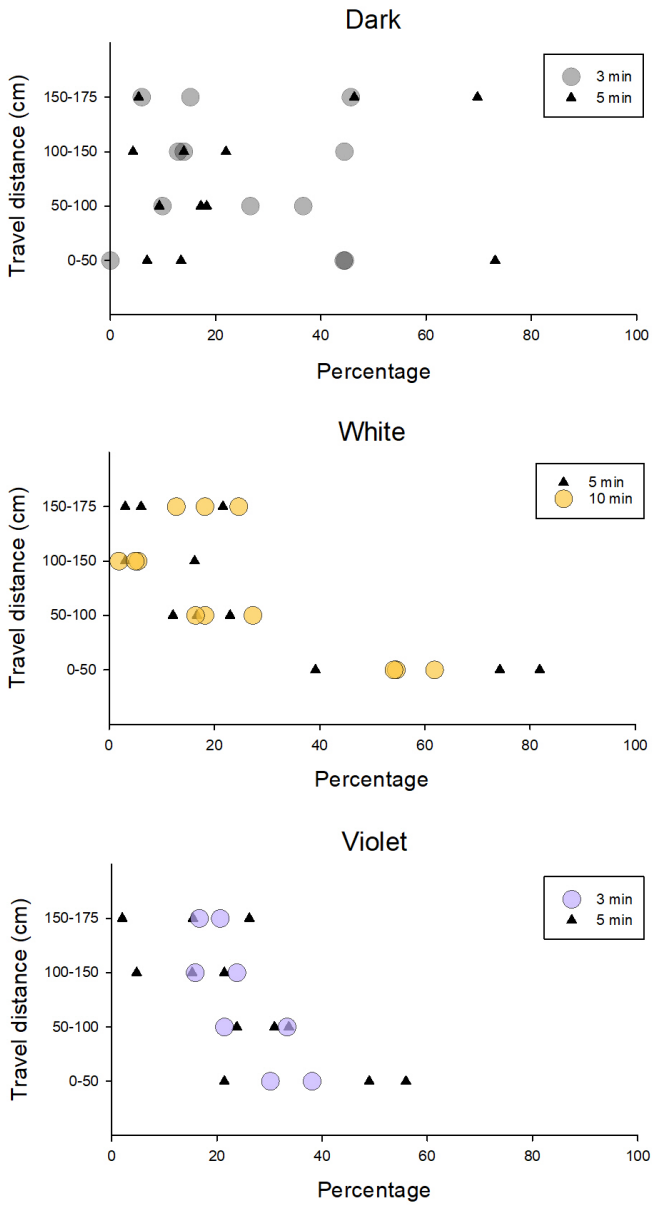

Figure 3.7: Relative travel distance of *Ls* Gulen nauplii in 4 sections of the experimental system at experiment end (n = 3, except 3 min violet (n = 2)). Experiment duration was either 3 (coloured circle), 5 (black triangle), or 10 (coloured circle) min.

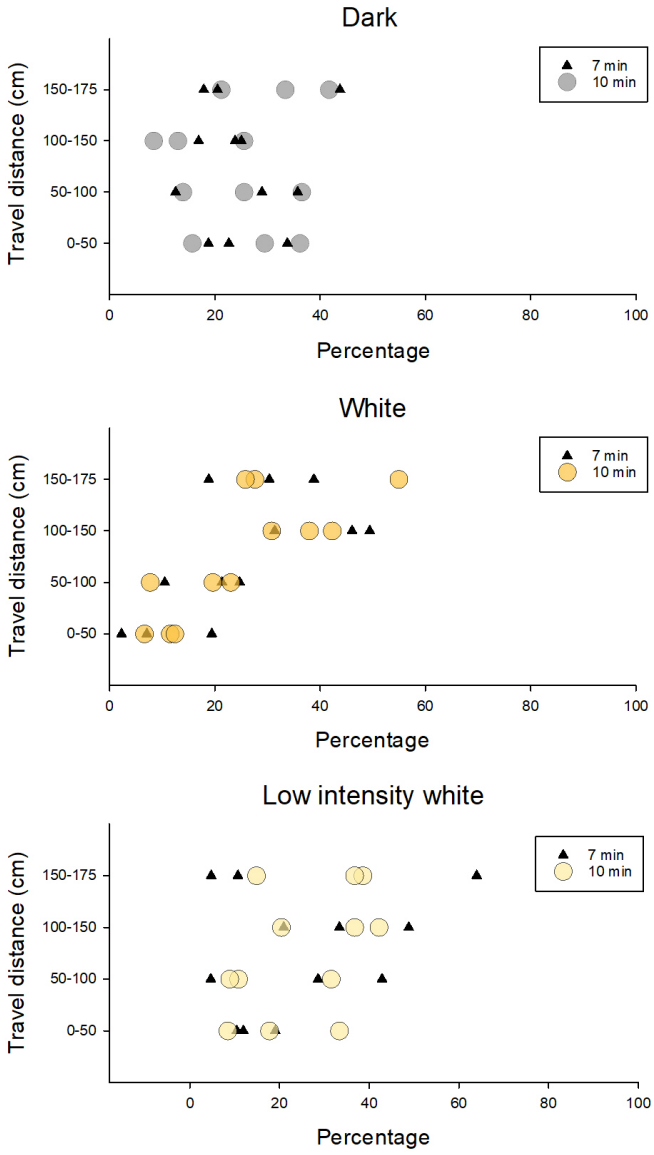

Figure 3.8: Relative travel distance of *Ls* Gulen copepodids in 4 sections of the experimental system at experiment end (n = 3). Experiment duration was either 7 (black triangle) or 10 (coloured circle) min.

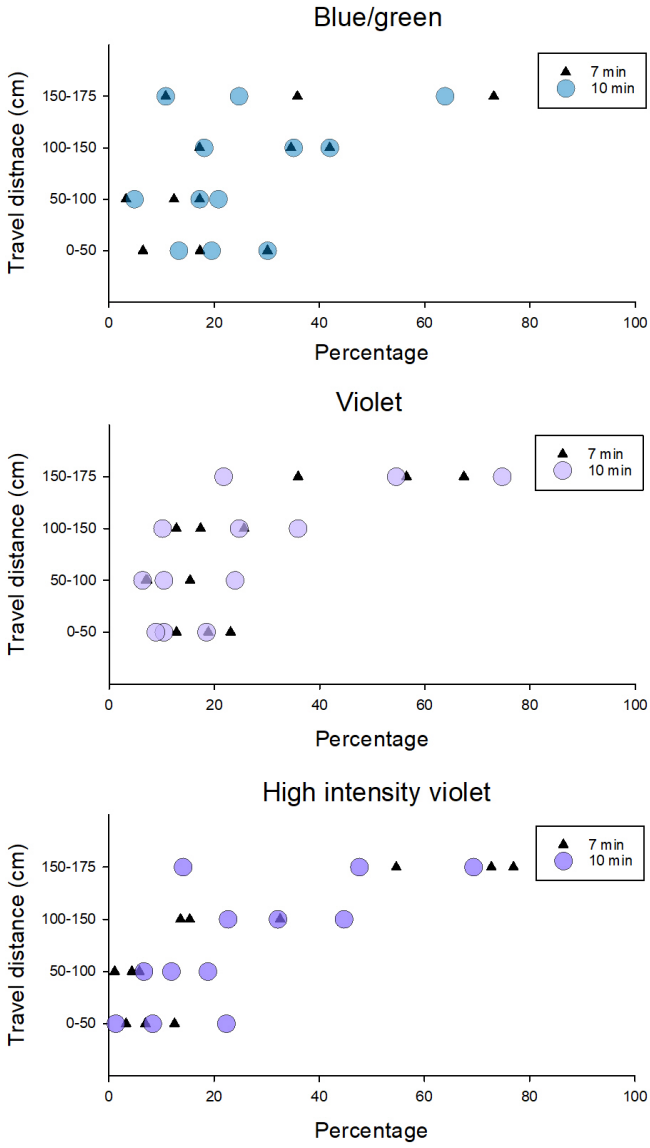

Figure 3.9: Relative travel distance of *Ls* Gulen copepodids in 4 sections of the experimental system at experiment end (n = 3). Experiment duration was either 7 (black triangle) or 10 (coloured circle) min.

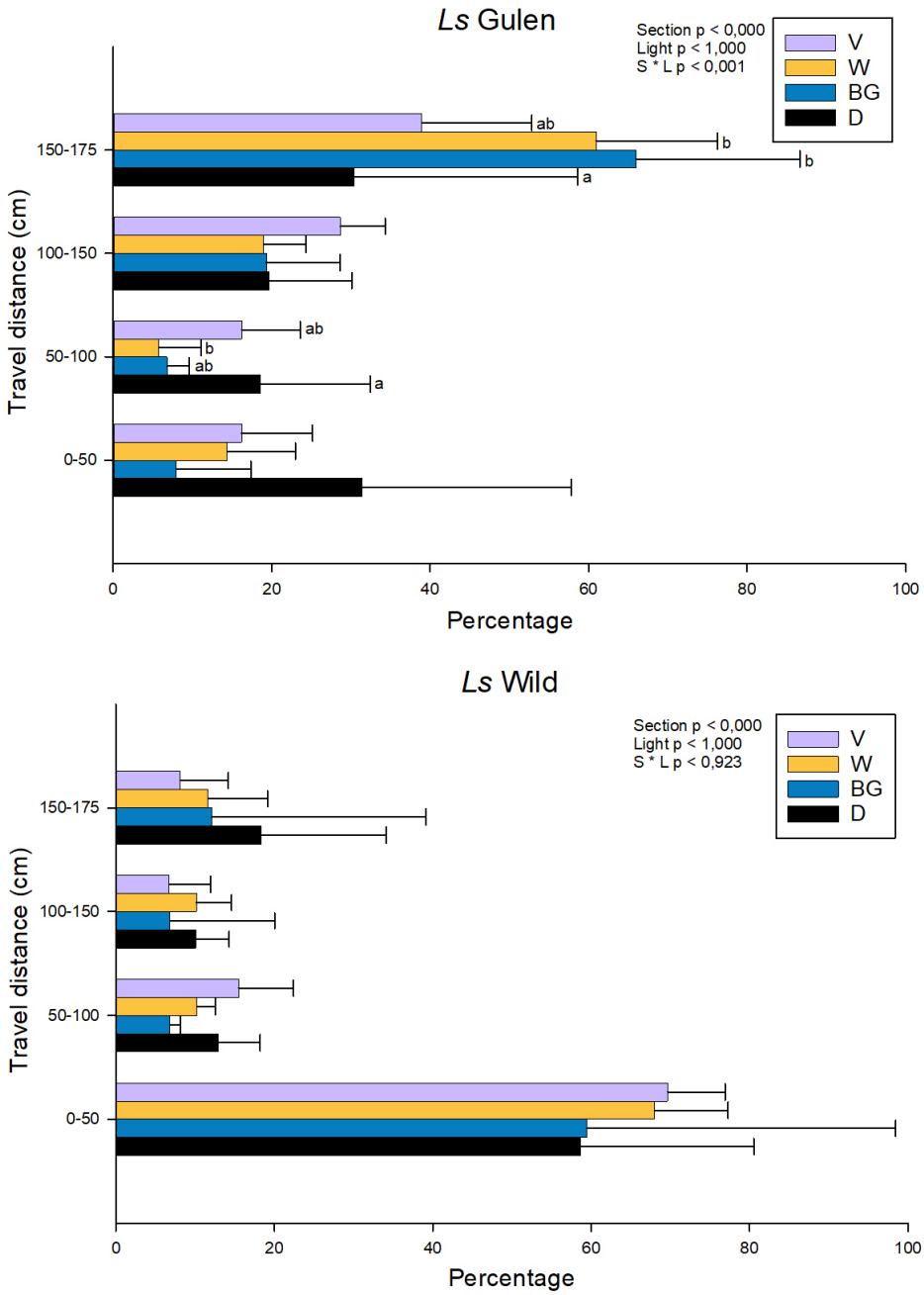

Figure 3.10: Relative travel distance of *Ls* Gulen (n = 6, except treatment V: n = 5) and Wild (n = 3) nauplii in the experimental system at experiment end: D) dark, BG) blue/green, W) white, V) violet. Standard deviation is shown with error bars and results from LSD analysis is shown with letters, denoting which treatments are considered significantly different from each other.

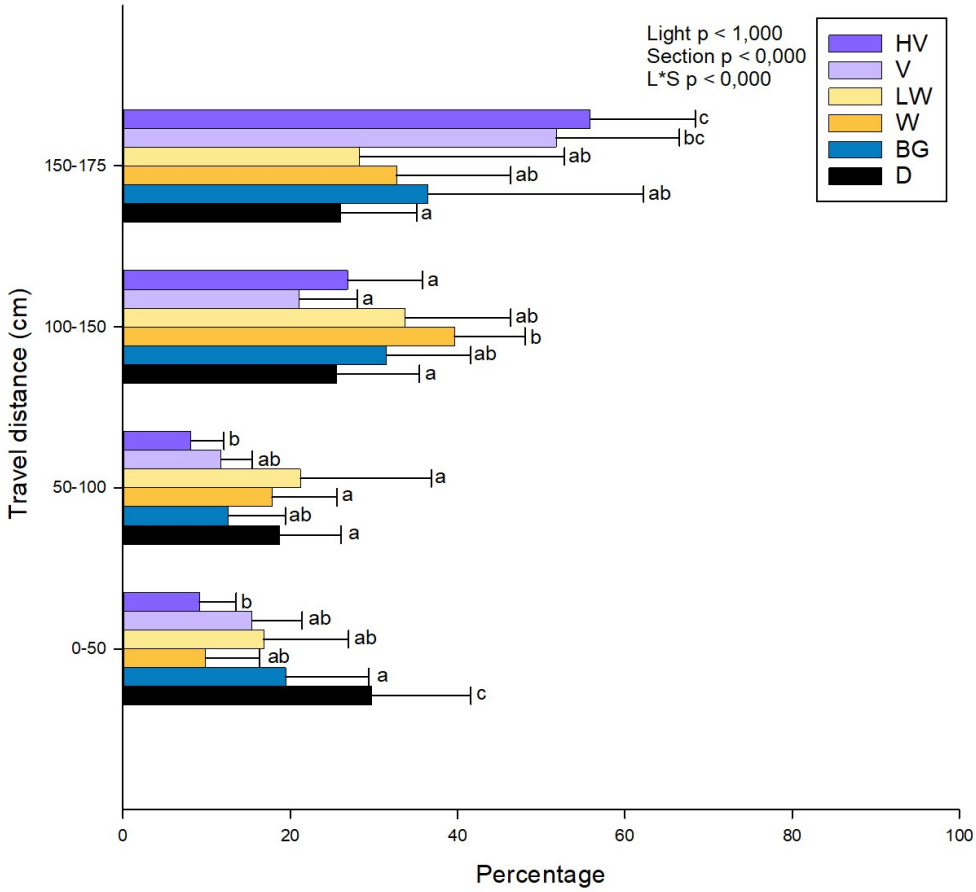

Figure 3.11: Relative travel distance of *Ls* Gulen copepodids in the experimental system at experiment end (n = 6): D) dark, BG) blue/green, W) white, V) violet, LW) low intensity white, HV) high intensity violet. SD is shown with error bars and results from LSD analysis is shown with letters, denoting which treatments are considered significantly different from each other.

Comparison of *Ls* Wild and Gulen

Two tailed t-tests analysing differences in phototactic response between the two nauplii strains found that very few *Ls* Gulen nauplii remained at origin (0-50 cm) compared to *Ls* Wild nauplii ($p < 0.05$) (Table 3.1, Figure 3.10). In general, *Ls* Gulen nauplii were more scattered throughout the upper part of the water column. White and violet light clearly attracted nauplii to the upper sections (100-150 cm: $p < 0.05$ and 0.003, respectively, 150-175 cm: $p < 0.001$ for both light wavebands), with concomitant depletion in the bottom section ($p < 0.001$ for both light wavebands). Due to highly variable migration of nauplii when exposed to BG light, there were found no statistically significant differences between the two strains was found.

Table 3.1: Results from two tailed t-tests between light treatments within each section for *Ls* Gulen and *Ls* Wild nauplii (95 % confidence interval).

Depth (cm)	Dark p-values	Blue/green p-values	White p-values	Violet p-values
150-175	0.440	0.092	**0.001**	**0.005**
100-150	0.093	0.487	**0.049**	**0.003**
50-100	0.411	0.989	0.120	0.894
0-50	**0.020**	0.143	**0.001**	**0.001**

Chapter 4

Discussion

Results from the field experiment indicate an increased density of salmon lice larvae inside the sea cage during daylight hours (DH) compared to darkness/twilight hours (DTH). Water current velocities in the area were much greater than known mean and maximum swimming velocities for nauplii and copepodids, which makes it unlikely that larvae are able to aggregate in the vicinity of artificial light. However, the range of emitted light from underwater lamps could extend beyond the boundaries of sea cages and cause salmon lice larvae to migrate towards fish farms.

Results from the laboratory experiment indicate an increased phototactic response to certain wavebands associated with commercial underwater lights. *Ls* Gulen nauplii had an increased response to blue/green (BG) and white (W) light, while *Ls* Wild nauplii had no significant response to any of the treatments. *Ls* Gulen copepodids had a positive phototactic response to violet (V) light.

4.1 Effect of light on concentration of salmon lice larvae in sea cages

Density of salmon lice larvae collected in plankton samples at Hosenøyan ranged from 0.0 to 1.8 larvae m^{-3}, with an average density of 0.3 larvae m^{-3}. These densities are normal lice densities at active salmon farms (Costelloe et al., 1996, 1998; Nelson et al., 2018). There were only found *L. salmonis* nauplii in visually analysed plankton samples, while developmental stage of larvae detected with ddPCR was undetermined. Genetic analysis with ddPCR is a much more efficient way to analyse plankton samples, but the method is not yet capable of discerning between nauplii and copepodids. Dimmen (2019) conducted a seasonal study on abundance of salmon lice larval stages at fish farm installations, where more than 99% of larvae were identified as nauplii. A different study, on horizontal and vertical distribution of *L. salmonis* larvae, found that 93% of captured larvae were nauplii (Nelson et al., 2018). As nauplii are much more abundant in

the water column in close proximity to salmon farms, most of the larvae detected by genetic analysis with ddPCR were likely nauplii.

No statistically significant ($p > 0.05$) variation in larval content was found in samples collected during DH compared to samples collected during DTH. Nevertheless, this does not mean that the effect of natural light is unrelated to larval density. Sample means and a low p-value are clear indicators of sunlight eliciting a biological response in salmon lice larvae. These findings are supported by earlier studies on DVM of *L. salmonis* larval stages, where larvae were found to aggregate in surface layers during DH and sink to deeper layers during DTH (Heuch et al., 1995; Nelson et al., 2018). A similar migration pattern (or reversed) has been observed for other crustacean and zooplankton species (Cushing, 1951; Forward, 1988).

One of the goals of the field study was to investigate the effect of artificial light on abundance of salmon lice larvae inside sea cages. Due to the duration of the field experiment being reduced from two weeks to two days, this was not possible and collected plankton samples could not be used to determine any effect of artificial light on larval concentration in the sea cage. We did, however, receive data on water currents from SKJERMTEK, and a discussion on *L. salmonis* swimming capacity and the effect of water current velocity follows.

4.1.1 Swimming capacity of *L. salmonis* larvae and the effect of water current velocity and direction

Mean horizontal current velocities during the field experiment ranged from 6 to 18 cm s^{-1}, with maximum velocities < 80 cm s^{-1}. Previous studies on the swimming behaviour of *L. salmonis* larvae have observed that both nauplii and copepodids exhibit positive geotaxis and therefore must expend energy on active, upward swimming, which is followed by passive sinking (Bron et al., 1993; Gravil, 1996). Often referred to as a "hop and sink" pattern. Copepodids swim faster and more frequently than nauplii, and have shorter rest periods in between (Bron et al., 1993). A study by Gravil (1996) concerning swimming velocities of salmon lice larvae recorded the mean swimming speed of nauplii to be 1.3 ± 0.2 cm s^{-1} and copepodids to be 2.1 ± 0.2 cm s^{-1}. A maximum speed of 10,2 cm s^{-1} was recorded for copepodids in the same study. Bjørnstad and Solstad (2019) used tracking to examine light response and swimming behaviour of copepodids and reported mean swimming speed to be below 0.4 cm s^{-1}, with maximum velocities around 10.0 cm s^{-1}. The lowest recorded mean current velocity was much greater than the highest average swimming velocity known for salmon lice larvae, and the maximum current velocity almost exceeded the highest registered copepodid swimming velocities by an order of magnitude. This implies that larvae would be swept away by water currents unless they managed to attach to a host.

Sea cages come in a multitude of sizes, but standard circumference of sea cages in Norway is either 100 or 160 m, which correspond to diameters of 32 and 51 m, respectively. Configuration of light systems depend on the dimensions of sea cages and the type of underwater lamps used, a common setup is between 3-5 powerful underwater lamps ($\sim$ 1000 W). Depending on spec-

tral composition of underwater lamps, and the kind and concentration of optically significant dissolved and particulate matter of seawater, emitted light could propagate over great distances under water. Modeled light of the underwater lamp used as a reference for the laboratory experiments had an output of 9.1 μmol photons m^{-2} s^{-1} at 20 m distance from the lamp and 1.9 μmol photons m^{-2} s^{-1} at 30 m distance from the lamp (calculated with diffuse attenuation coefficients from data measurements at Hosenøyan). These are intensities known to be of biological relevance to salmon lice larvae, which have been found to respond to intensities as low as 1.6 μmol photons m^{-2} s^{-1} and 0.057 μmol photons m^{-2} s^{-1} (nauplii and copepodids, respectively) (Gravil, 1996). Thus, current light systems have the potential to emit light beyond the circumference of sea cages, which could attract salmon lice towards the fish farm. However, emission of light will be disturbed by the fish themselves and by the net wall and biofouling it carries. In addition, water current velocity and direction has a great impact on directional movement of larvae.

4.2 Light response of salmon lice larvae to light of different wavelengths

4.2.1 The effect of time on travel distance

An analysis of the effect of time on travel distance, for both *Ls* Gulen nauplii and copepodids, revealed that there was no significant effect of time ($p > 0.05$ for both stages) for the different treatments. Gravil (1996) discovered that larvae swim both in light and darkness, and that active swimming almost always happens in an upward direction. Periods of passive sinking followed active swimming, and were usually longer in duration. However, the distance covered during active swimming was always greater than the distance larvae sank, resulting in a net advance upward. Results from the present study could indicate that majority of larvae in experiments reached their final destination in the system early, and that they may have found a surface to adhere to or remained relatively stationary in the water column with their "hop and sink" swimming pattern. For copepodids, results could indicate that acclimatization to darkness had no effect on their phototactic behaviour, and that the assumption of increased response time (similar to nauplii response, Section 2.2.5) was incorrect.

4.2.2 Wavelength-specific behaviour

In general, *Ls* Gulen nauplii and copepodids had a tendency to move from the bottom section to other sections of the system with or without light stimuli, while majority of *Ls* Wild nauplii remained in the bottom section (0-50 cm) for all treatments. Due to a highly variable phototactic response to BG light, no statistical significance was found between the control treatment (D) and BG light for *Ls* Wild nauplii or *Ls* Gulen copepodids. However, LSD analyses revealed that the percentage of *Ls* Gulen nauplii in the top section for the BG treatment was significantly

different from the control treatment. In addition, increased positive phototactic response of *Ls* Gulen copepodids to violet light was found.

Light influences distribution of *L. salmonis* larval stages in their natural environment (Heuch et al., 1995), thus, a logical assumption would be to assume that their visual spectral sensitivity is adapted to the spectrum of light under water. Visible light in the violet and, especially, blue spectrum penetrate deepest in the clearest ocean waters, with 1% of the light reaching depths down to 300 m (Sakshaug et al., 2016) (Figure 4.1). In coastal waters, where phytoplankton and CDOM content often is high (Figure 1.2), light in the green spectrum (500-550 nm) penetrates deepest. DVM of *L. salmonis* larvae is controlled by light and characterized by an ascent to minimum depth during the day and descent to maximum depth at night (Heuch et al., 1995). At twilight, the downwelling light has a peak in the blue/green spectral region, thus, sensitivity to blue/green light would be beneficial for *L. salmonis* larvae in relation to DVM (Forward, 1988). Gravil (1996) found *L. salmonis* nauplii to have a peak positive phototactic response to light of 500 nm and copepodids to have a peak response to wavelengths between 500-561 nm, with a broad range of sensitivity between 430-700 nm. Similar results for copepodids were discovered by Bron et al. (1993), with a peak response to light of 550 nm, a fairly uniform response to light between 500-700 nm, and a low response to light of 400 nm. Light colours and intensities used in the present study were selected for biological relevance, and simulated the spectral distribution of an underwater lamp commercially used in aquaculture today. Ranging from 0.24-23 μmol photons m^{-2} s^{-1}, selected intensities confound a direct comparison of wavelength-specific behaviour in larvae, as light intensity also influences larval response. High intensity could confuse and overwhelm copepodids, which have a higher sensitivity to light than nauplii (Flamarique et al., 2000), causing a variable response to the BG light treatment. The contrasting response of nauplii strains (*Ls* Gulen and Wild) could be caused by genetic dissimilarities or different rearing conditions.

4.2.3 Behaviour related to lice strains, experimental design and rearing conditions

Significant differences in light response was found between *Ls* Gulen and Wild nauplii, with *Ls* Gulen nauplii showing a greater phototactic response to BG and W light than *Ls* Wild nauplii. *Ls* Gulen nauplii (and copepodids) came from a strain that had been reared in a laboratory for more than 70 generations, while *Ls* Wild nauplii had been collected from wild Atlantic salmon. A study analysing energy consumption of salmon lice larvae found that *Ls* Gulen copepodids had lower energy reserves than copepodids collected from farmed Atlantic salmon 14 days post hatching (0.015 vs. 0.030 cal) (Fotland, 2019). It is possible that salmon lice reared in laboratories for generations have a different genetic makeup and response to sensory cues, which could account for some of the differences in light response between both *Ls* Gulen and Wild nauplii, and between copepodids used in experiments of the present study and previous studies by different authors (Bron et al., 1993; Gravil, 1996).

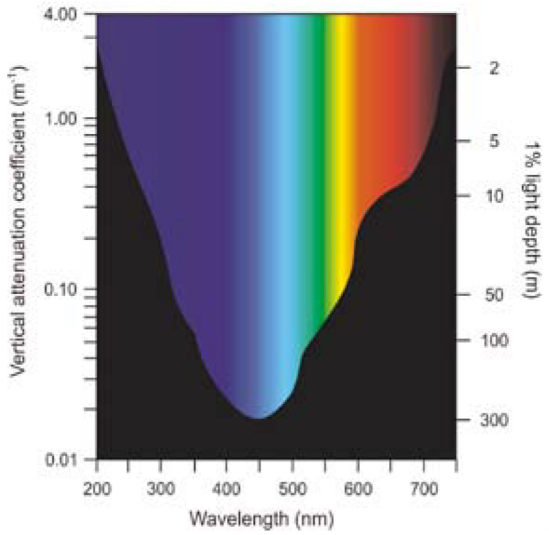

Figure 4.1: Vertical diffuse attenuation coefficients for different wavelengths in clearest ocean water. Modified from Sakshaug et al. (2016); illustration by Zsolt Volent.

In choosing a vertical experimental system, gravity was introduced as a factor which influences salmon lice larvae. Results from previous studies (Bron et al., 1993; Gravil, 1996) were found using horizontal systems that attempted to isolate behaviour related to light wavebands. In a natural environment, salmon lice larvae are constantly experiencing nuances in pressure, which could play a key role in influencing the behaviour of larvae (Brooker et al., 2018). Thus, larvae in vertical systems could behave differently to light stimuli than larvae in horizontal systems.

Water flow in the hatchery seemed to have a huge impact on larval viability. *Ls* Gulen nauplii and copepodids were reared in an environment with high water flow (38 mL min^{-1}), whereas *Ls* Wild nauplii were reared in an environment with low water flow (3 mL min^{-1}). Larvae exposed to high water flow had difficulties moulting from nauplii to copepodids and were observed lying at the bottom of incubator wells, unable to shed their old exoskeleton. Larvae that managed to moult successfully were likely experiencing some adverse effects due to being reared in an environment with high water flow, which could have influenced their behaviour and response to light in the experimental system.

4.3 Challenges and limitations

4.3.1 Field experiment

The experimental period was too short to obtain enough replicates for proper analyses of time series data, especially considering one would need enough replicates for time series data both with

and without artificial light to answer the hypothesis related to the field experiment. Completing the experiment earlier in the autumn would also have been beneficial, as the concentration of salmon lice larvae generally is higher during September/October and there also is sufficient darkness to investigate the effect of artificial light. In this context, a future field study with longer duration would bear merit and could provide interesting results on the topic.

Developmental stage of larvae in samples analysed genetically with ddPCR could not be determined, it would have been helpful to analyse samples both visually and genetically to find out whether these larvae were nauplii or copepodids.

4.3.2 Laboratory experiment

Complications with the hatchery led to reduced viability of salmon lice larvae, resolving these issues took a lot of time that could have been spent refining methodology and completing more experiments. Initially, additional experiments with varying light intensities and inclusion of halide light were intended, as well as a more comprehensive comparison of different *L. salmonis* strains.

Results from experiments with *Ls* Gulen larvae have an added insecurity due to reduced viability of larvae caused by rearing conditions. These complications also made it difficult to find an appropriate experimental duration for both nauplii and copepodids, as their response varied greatly depending on larval viability.

Chapter 5

Conclusions

The present study could not conclude whether artificial light influences concentration of salmon lice larvae inside sea cages. Measurements of ocean currents made it clear that swimming capacities of salmon lice larvae are too limited for them to be able to aggregate in the vicinity of underwater lights. However, light emitted by underwater lamps can exceed the circumference of sea cages and potentially elicit a positive phototactic response in larvae, causing them to swim towards the fish farm.

Light in the blue/green (BG) spectrum was only found to cause an increased positive phototactic response in *Ls* Gulen nauplii. However, both nauplii strains and copepodids had a highly variable response to BG light between replicates. The variable response could possibly be explained by the high intensity of the light, which could have overwhelmed and confused the larvae. Contrasting response between the two nauplii strains could be due to differences in rearing conditions, but could also be caused by genetic dissimilarities.

Ls Gulen copepodids showed an increased response to violet light. Use of violet light in sea cages could potentially attract copepodids to the fish farm, however, light in the violet spectrum is absorbed by CDOM and phytoplankton, and usually does not propagate far in coastal water.

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Appendix A

Water volume of plankton samples

Table A.1: Water volume of plankton samples.

Sample #	Tow #	Flow start	Flow stop	# of revolutions	Volume (m^3)	**Sample volume (m^3)**
1	1	37086	37116	30	2.250	**8.775**
1	2	37028	37115	87	6.525	
2	1	37163	37193	30	2.250	**5.100**
2	2	37209	37247	38	2.850	
3	1	37258	37289	31	2.325	**4.425**
3	2	37298	37326	28	2.100	
4	1	37339	37384	45	3.375	**4.950**
4	2	37392	37413	21	1.575	
5	1	37427	37484	57	4.275	**6.300**
5	2	37463	37490	27	2.025	
6	1	37503	37534	31	2.325	**6.300**
6	2	37506	37559	53	3.975	
7	1	37619	37642	23	1.725	**3.675**
7	2	37645	37671	26	1.950	
8	1	37678	37702	24	1.800	**3.600**
8	2	37712	37736	24	1.800	
9	1	37742	37765	23	1.725	**3.450**
9	2	37776	37799	23	1.725	
10	1	37807	37831	24	1.800	**3.600**
10	2	37838	37862	24	1.800	

Table A.2: Water volume of plankton samples.

Sample #	Tow #	Flow start	Flow stop	# of revolutions	Volume (m^3)	**Sample volume (m^3)**
11	1	37878	37902	24	1.800	**3.750**
11	2	37904	37930	26	1.950	
12	1	37932	37967	35	2.625	**4.650**
12	2	37969	37996	27	2.025	
13	1	38001	38026	25	1.875	**3.900**
13	2	38035	38062	27	2.025	
14	1	38070	38088	18	1.350	**3.225**
14	2	38095	38120	25	1.875	
15	1	38159	38172	13	0.975	**2.175**
15	2	38178	38194	16	1.200	
16	1	38200	38222	22	1.650	**2.925**
16	2	38226	38243	17	1.275	
17	1	38248	38267	19	1.425	**2.550**
17	2	38275	38290	15	1.125	
18	1	38299	38321	22	1.650	**3.000**
18	2	38329	38347	18	1.350	
19	1	38443	38453	10	0.750	**1.650**
19	2	38460	38472	12	0.900	
20	1	38489	38492	3	0.225	**1.275**
20	2	38496	38510	14	1.050	
21	1	38516	38548	32	2.400	**4.575**
21	2	38555	38584	29	2.175	
22	1	38592	38615	23	1.725	**3.600**
22	2	38673	38698	25	1.875	
23	1	38704	38720	16	1.200	**3.150**
23	2	38724	38750	26	1.950	
24	1	38758	38785	27	2.025	**3.825**
24	2	38789	38813	24	1.800	
25	1	38821	38847	26	1.950	**3.675**
25	2	38853	38876	23	1.725	
26	1	38881	38896	15	1.125	**2.625**
26	2	38902	38922	20	1.500	
27	1	38931	38954	23	1.725	**3.750**
27	2	38961	38988	27	2.025	
28	1	38993	39015	22	1.650	**3.150**
28	2	39019	39039	20	1.500	
29	1	39045	39067	22	1.650	**3.300**
29	2	39074	39096	22	1.650	
30	1	39104	39113	9	0.675	**2.325**
30	2	39120	39142	22	1.650	

Table A.3: Water volume of plankton samples.

Sample #	Tow #	Flow start	Flow stop	# of revolutions	Volume (m^3)	**Sample volume (m^3)**
31	1	39148	39172	24	1.800	**3.750**
31	2	39178	39204	26	1.950	
32	1	39211	39235	24	1.800	**3.225**
32	2	39241	39260	19	1.425	
33	1	39273	39298	25	1.875	**3.900**
33	2	39304	39331	27	2.025	
34	1	39335	39363	28	2.100	**3.825**
34	2	39369	39392	23	1.725	
35	1	39402	39432	30	2.250	**4.500**
35	2	39438	39468	30	2.250	
36	1	39476	39504	28	2.100	**3.975**
36	2	39510	39535	25	1.875	
37	1	39541	39559	18	1.350	**3.075**
37	2	39565	39588	23	1.725	
38	1	39593	39615	22	1.650	**3.000**
38	2	39620	39638	18	1.350	
39	1	39643	39667	24	1.800	**2.925**
39	2	39701	39716	15	1.125	
40	1	39726	39740	14	1.050	**3.000**
40	2	39745	39771	26	1.950	

Appendix B

Water volume and larval content of plankton samples

Table B.1: Water volume, salmon lice larvae and calculated larvae m^{-3} for plankton samples collected inside sea cage 5 at Hosenøyan during daylight hours (DH) and darkness/twilight hours (DTH).

Sample #	Sample volume (m^3)	# of larvae	Larvae per m^3	Light conditions
1	8.775	0	0.0	DTH
2	5.100	0	0.0	DTH
3	4.425	1	0.2	DTH
4	4.950	2	0.4	DH
5	6.300	2	0.3	DH
6	6.300	0	0.0	DH
7	3.675	0	0.0	DH
8	3.600	1	0.3	DTH
9	3.450	1	0.3	DTH
10	3.600	1	0.3	DTH
11	3.750	4	1.1	DTH
12	4.650	1	0.2	DTH
13	3.900	7	1.8	DTH
14	3.225	1	0.3	DH
15	2.175	0	0.0	DH
16	2.925	1	0.3	DH
17	2.550	0	0.0	DH
18	3.000	0	0.0	DH
19	1.650	1	0.6	DH
20	1.275	0	0.0	DH
21	4.575	1	0.2	DH
22	3.600	1	0.3	DH
23	3.150	2	0.6	DH
24	3.825	1	0.3	DTH
25	3.675	0	0.0	DTH
26	2.625	1	0.4	DTH
27	3.750	1	0.3	DTH
28	3.150	1	0.3	DTH
29	3.300	3	0.9	DTH
30	2.325	0	0.0	DTH
31	3.750	1	0.3	DTH
32	3.225	2	0.6	DTH
33	3.900	2	0.5	DTH
34	3.825	2	0.5	DTH
35	4.500	1	0.2	DH
36	3.975	1	0.3	DH
37	3.075	1	0.3	DH
38	3.000	0	0.0	DH
39	2.925	0	0.0	DH
40	3.000	1	0.3	DH

Appendix C

Shapiro-Wilk tests and distribution of data

Results from Shapiro-Wilk tests show that data from the field experiment was not normally distributed ($p < 0.006$) (Table C.1), but had a Poisson distribution (Figure C.1).

C.1 Field Experiment

Table C.1: Results from Shapiro-Wilk tests to see if data was normally distributed for field exp: DH) daylight hours, DTH) darkness/twiight hours.

Light conditions	Statistic	df	Sig.
DH	0.853	20	0.006
DTH	0.782	20	0.000

C.2 Laboratory experiment

Results from Shapiro-Wilk tests show that data from the laboratory experiment was not normally distributed ($p < 0.001$) (Table C.2), but had a Poisson distribution (Figure C.2).

Table C.2: Results from Shapiro-Wilk tests to see if data was normally distributed for lab exp.

Lice group	Statistic	df	Sig.
Ls Gulen nauplii	0.878	80	0.000
Ls Wild nauplii	0.752	48	0.000
Ls Gulen copepodids	0.910	152	0.000

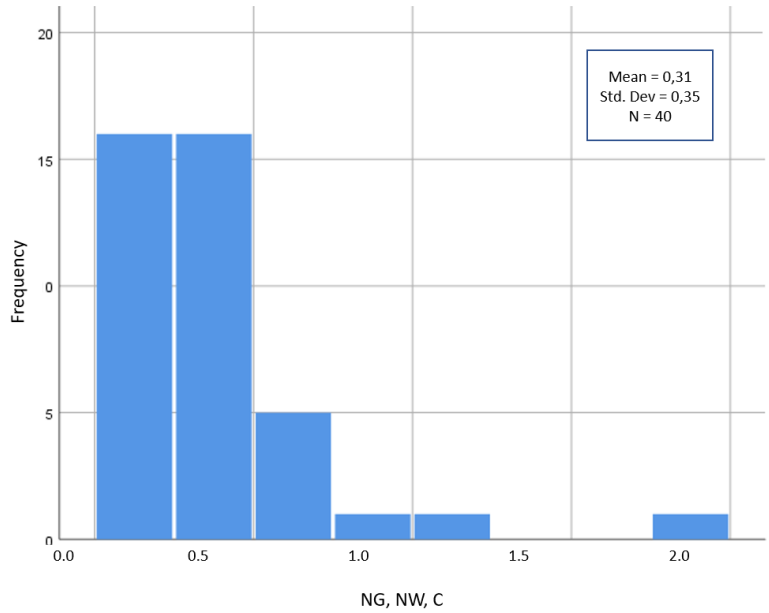

Figure C.1: Histogram of data residuals for field exp.: NG) *Ls* Gulen nauplii, NW) *Ls* Wild nauplii, C) copepodids.

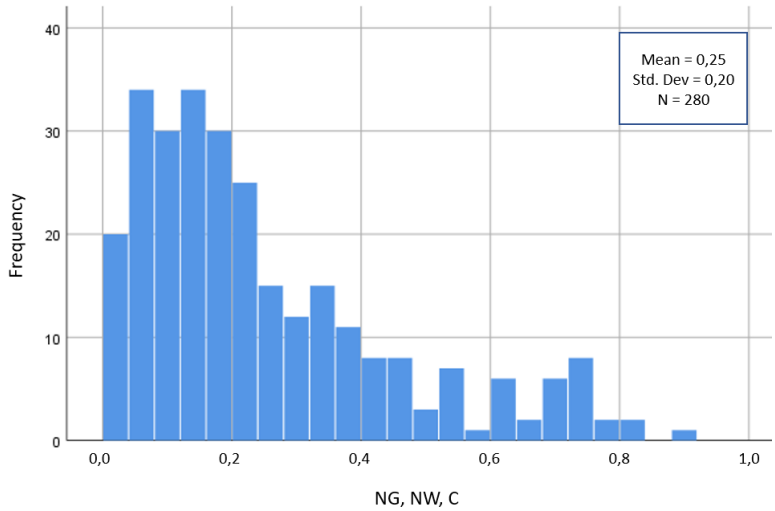

Figure C.2: Histogram of data residuals from lab exp.: NG) *Ls* Gulen nauplii, NW) *Ls* Wild nauplii, C) copepodids.