Temperature-dependent development of embryonic, planktonic, and parasitic stages of the sea lice *Caligus elongatus*

Gine Myhre

Master's Thesis in Aquaculture and Seafood

Department of Biosciences
THE UNIVERSITY IN BERGEN

September 2021

Acknowledgement

I want to thank IMR Matre for financing this project. I would like to thank my Supervisor Samantha Bui for helping me with all the practical setups in the trials, for always being available on the phone and for guiding me through the months of writing. Thank you, Lars A. Hamre and Sussie Dalvin, for helping me out with the trials and for guiding me and reviewing my thesis. Thank you, Aina-Cathrine Øvergård, for assistance and great feedback on my thesis. I would also give a big thank you to Lise Dyrhovden for helping me out with the control system and for many good talks. I want to thank the whole staff at IMR Matre for helping me with all the practical work and for bringing so much joy and new knowledge into my life.

I want to give a huge thanks to my fellow students Henrikke B. Oppedal and Malin Stalheim for assistance and for supporting me through these 5 years. I want to thank my family for being supportive and understanding. Thank you, Martin Orre, for encouraging me all these years and especially through these last months before submission.

Bergen
September 2021
Gine Myhre

Table of Contents

Abstract

The sea louse *Caligus elongatus* is an important ectoparasite which infects a wide range of fish species in the sea. However, the most studied sea lice are *Lepeophtheirus salmonis* (the salmon lice), including how their development rate is affected with changing seawater temperatures. With the rising abundance of *C. elongatus* at salmon farms in Norway, there is a need to describe the development rate of *C. elongatus* in different temperatures, which would facilitate management strategies. In this study the embryonic, planktonic, and parasitic developmental stages of *C. elongatus* was examined under laboratory conditions. Atlantic salmon (*Salmo salar*) kept in full-salinity seawater were used as experimental host fish. The observations of the lice were made at 6, 9, 12 and 15°C to determine temperature-dependent development rates, and the full experiment consisted of two parts: in part 1, the fish were infected in tanks holding 10 fish each, by offspring of several *C. elongatus* females. The hosts were lightly sedated before sampling at consecutive time periods after infection to capture the time until molt into the adult stage of the parasite. In part 2, the focus was on the eggs of *C. elongatus*, whereby these would be further incubated to study the embryonic and planktonic development. Lastly, the copepodid survival would be observed of all the different experimental temperatures. Results indicates that temperature significantly affects both the developmental stages and the morphometry of *C. elongatus*, in which the lice develop slower, survive longer and are bigger with colder temperatures and develop faster, survive shorter and are smaller with warmer temperatures.

1 Introduction

1.1 Fish and Parasites

Fish have a history that extends around 500 million years and were one of the first vertebrae on earth. Closely related to the evolution of fishes, we find the evolution of invertebrates which include parasites on fish. These species have coevolved and adapted to each other's defenses, and this relationship is termed host and parasite (Carrera et al., 1998). A parasite is an organism that lives in or on another living organism, occupies parts of the nutrition from the host, shows a certain degree of adaption to the host and causes some damage to the host (Karlsbakk et al., 2019). The parasite can affect the host in several ways, such as destroying cells or tissue, by excreting toxic substances, by making it easier for other pathogenic organisms to penetrate or by influencing the immune system of the host (Karlsbakk et al., 2019).

1.2 Sea lice (Caligidae)

Sea lice are a freshwater and marine parasite that spend several life cycle stages attached to the exterior of their host (an ectoparasite). Broadly, sea lice have a range of hosts as wide as the number of fish species existent. Some lice are called specialists and will only parasitize on one or a few certain hosts, examples include salmon, halibut, and turbot lice. Other lice have a low degree of host specificity and are called generalists. *Caligus elongatus* is a generalist and has been found on around 80 different hosts (Heuch & Schram, 1999). Salmon lice and *C. elongatus* are members of the class Copepoda and the family Caligidae, and both are ectoparasites living on the surface of the fish skin.

Sea lice is a collective concept term within the fish farming industry, which covers several species of ectoparasitic crustaceans. There are several different species of Caligus infecting farmed salmonids, and *C. elongatus* is most common in the North Atlantic, *C. orientalis* and *C. clemensi* in the North Pacific, and *C. rogercresseyi* and *C. teres* in Chile (Hemmingsen et al., 2020). Overall, at least 267 species of Caligus has been described (Walter & Boxshall, 2021). *C. elongatus* was first described by Nordmann (1832), but he had some major errors in his description. Parker (Parker, 1969) then made a new improved description. Some researchers that have studied the life cycle of *C. elongatus* are Hogans & Trudeau, 1989; Piasecki & Mackinnon, 1995; Piasecki & MacKinnon, 1993; Pike et al., 1993, and a morphological description of the whole life cycle came when Piasecki (1996) described and illustrated all eight development stages of *C. elongatus*.

1.3 Fish farming industry

The fish farming industry in Norway had its early beginning in the 1960s has expanded its production ever since. The large production of mainly Atlantic salmon and other salmonids has had a big impact on the number of parasites along the coast of Norway. More hosts available equals more parasites. The parasites with the biggest impact in Norwegian fish farming is the salmon louse *Lepeophtheirus salmonis* (Krøyer). In addition, the sea lice *Caligus elongatus* (Nordmann) has caused problems in some areas in Norway and in Scotland. Sea lice have had a major impact on the economy of the aquaculture sector and has been a controlling factor of the production of fish in the sea. All current legislation regarding the control of lice in Norwegian fish farming is aimed at the salmon lice (Heuch & Schram, 1999). The legislation (*Forskrift om bekjempelse av lakselus i akvakulturanlegg - Lovdata*, n.d.) was formed due to high sea lice infestations, with the aim to protect the wild populations of salmonids. This law determines the number of adult female lice there can be per farmed fish. The maximum mean number of allowed sexually matured female lice is 0.5, and in the period where the smolt migrates to the sea, the number is adjusted down to a maximum of 0.2 sexually mature female lice per fish.

A traffic light system was then formed by an expert team based on the reported abundance of salmon lice and other environmental impacts from lice. The expert team consists of independent researchers with a broad knowledge that covers all of the information regarding biology of sea lice, fish, ocean currents and other environmental factors that was needed before assembling this system (*Trafikklyssystemet – HI sin kunnskap | Havforskningsinstituttet*, n.d.). A connectivity analysis was conducted of the ocean currents to naturally divide the coastal areas into 13 different zones for fish farming. Each zone is evaluated every second year, which decides the next two years for farming facilities in that zone. In green zones it is assumed that under 10 % of the wild salmon smolt mortality occurs due to salmon lice, and farms in that zone are allowed to increase their production with 6 %. In yellow zones, it is assumed that 10-30 % of the salmon smolt die due to salmon lice, and farms cannot increase their production but can continue with the same production as before. In red zones, it is assumed that over 30 % of the salmon smolt die due to salmon lice and biomass production in the zone must be reduced by 6 %.

The law regarding sea lice infestations currently considers the level of salmon lice (*L. salmonis*), while *C. elongatus* infestations are not yet required to be reported to the Norwegian Food Safety Authority. On the other hand, in the recent years it has been reported

of large numbers of *C. elongatus* on Atlantic salmon in Northern Norway (Hemmingsen et al., 2020), which could have various of reasons that will be discussed in the later sections.

1.3.1 Control of the Sea lice

Since the beginning of the fish farming industry, there has been put in a great amount of time and money into fighting the sea lice. The different methods for controlling the sea lice infestations can be divided into three categories: chemical, preventive, and biological methods.

Chemical methods

In the early start of fish farming different chemicals such as formalin and acetic acid baths were used. The different compounds that has been used is orally administrated avermectins (emamectin benzoate) and benzoyl ureas (diflubenzuron and teflubenzuron), or bath treatments, using organophosphates (azamtiphos), pyrethroids (deltametrin and cypermetrin) and disinfectants (hydrogen peroxide) (Aaen et al., 2015). This has primarily been used to fight of the salmon lice, but Wootten et al. (1982) found that the effect of using a type of chemotherapy called Dichlorvos had the similar effect on both *C. elongatus* and *L. salmonis*. These treatments might be effective, but they carry an environmental risk and is affecting other non-target organisms. Another negative side is that the treatments can build a resistance in the lice against the chemical treatments (Hemmingsen et al., 2020). Environmentally friendly methods are therefore highly needed.

Preventive methods

The preventive methods incorporate different physical barriers of keeping the lice apart from the farmed fish. Various designs of plankton nets and tarpaulin skirts have been used and has been proved effective against the salmon louse (L. H. Stien et al., 2012). A more recent development is the “snorkel” cage which lowers the whole sea cage deeper than where the sea lice are. The cage has a tube up to the surface for the fish to swim up and refill their swim bladder. This separates the fish and louse physically and has been proved to be highly effective against salmon louse (Geitung et al., 2019). However, it is not investigated how this works for *C. elongatus* but since there are evidence that this louse can occur deeper in the water column that the salmon louse (á Nordi et al., 2015), and a lice skirt might not be able to prevent *C. elongatus* from infecting the farmed fish.

Biological methods

These methods include the use of cleaner fish, vaccination, selective breeding, and fish behavior. The cleaner fish that is used for lice control in the northern hemisphere are ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*). Wrasse is proven to be an effective cleaner but less effective in colder temperatures (Powell et al., 2018). Lumpfish works better in cold waters and will feed on both *L. salmonis* and *C. elongatus.* One disadvantage is that lumpfish has been shown to be a favorable host for *C. elongatus*. Another disadvantage is that lumpfish is an opportunistic feeder and will eat other food sources before they eat the sea lice (Hemmingsen et al., 2020). Overton et al. (2020) gathered all evidence base over the use of cleaner fish as a removal tool for sea lice. Their conclusion was that there is a mismatch between the evidence base that now exists around the use of cleaner fish and the actual number of cleaner fish that are being used inside fish farms for lice removal. They also add that the evidence is based on quite few studies and the studies that exists is in a small scale compared to how they are being used in the fish farm pens. Therefore, there is a great need for a more solid evidence base that can argue that the use of cleaner fish is ethically, environmentally, and economically correct.

1.4 Life cycle of *Caligus elongatus*

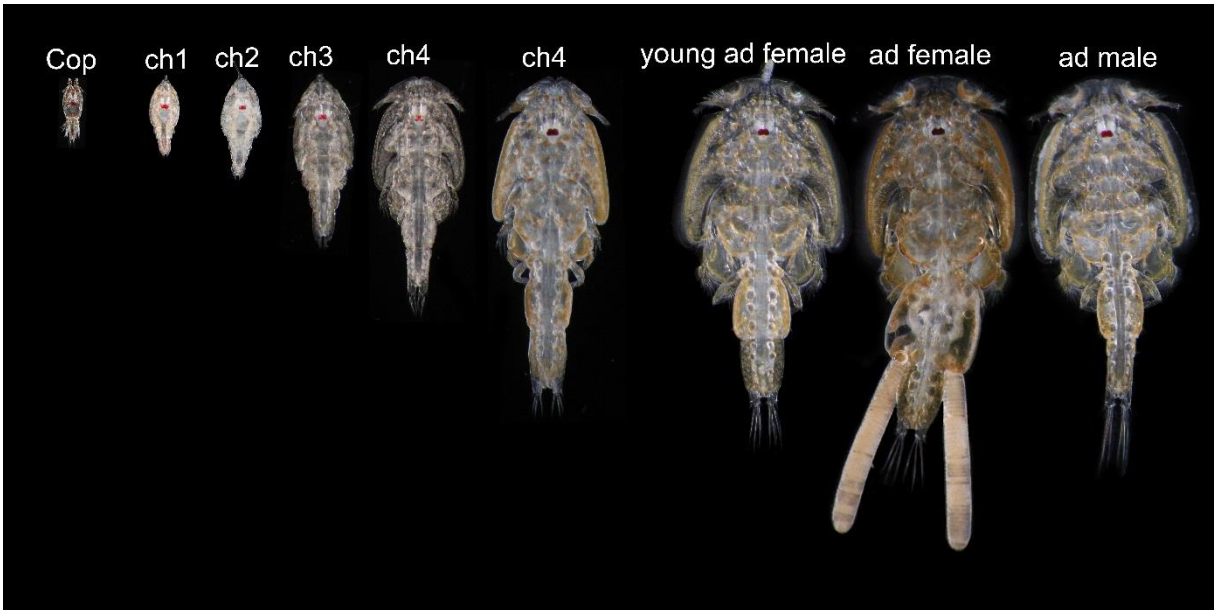

Fig. 1.4: The life cycle stages of Caligus elongatus; cop: copepodid, ch1: chalimus 1, ch2: chalimus 2, ch3: chalimus 3, ch4: chalimus 4, ad: adult. (Photo: Lars A. Hamre)

1.4.1 Developmental stages

C. elongatus have a direct life cycle with eight stages (Fig. 1.4). The larvae hatch directly from the egg strings into the water. There are two nauplii stages, nauplius I and nauplius II. The next stage is called the copepodid and is the stage where the lice is infective and attaches to the fish with grasping appendages (2. antennae) and later with a chitin tread (frontal filament). The lice will go through another molt and become chalimus I. There are four chalimus stages (I-IV) and these are also called sessile stages because they are attached to the fish with a frontal filament and do not move around the surface of the host. Chalimus IV molts into adult with a suction-cup shaped cephalothorax, allowing them to break free from the filament and move freely over the host surface (Heuch & Schram, 1999; Piasecki, 1996; Piasecki & Mackinnon, 1995).

Nauplius I and II

The first stage, after hatching, is called nauplius I and swims freely in the water column, using its three pairs of appendages (Piasecki & Mackinnon, 1995). The time it takes to molt into nauplius II is temperature-dependent but under 10 °C it can take around 24 hours (Piasecki & Mackinnon, 1995). Newly hatched nauplius I have a form that reflects the shape of the egg, a short cylinder, which will become elongated with time. Both stages have an oval form, where the anterior part is the widest. This is also where the antennas are situated. The larvae have a precursor to a mouth. The first pair of antennas are uniramous and consists of two segments and has several setae. Second and third pair of antennas are biramous and consists of several segments and has several setae. The third pair of antennas are called mandibles. Posterior on the lice there are two unsegmented, rodlike structures, called balancers. In nauplius II, the antennas are situated further posterior, and in some older individuals the copepodid stage is visible on the inside of the tegument of the lice (Piasecki, 1996).

Both nauplius I and II are positively phototactic and uses their brushed antennas to swim towards the light, to help them stay in the upper water layers. The nauplius larvae are not infective, but it is practical for them to stay close to a potential host to increase the chance for the copepodid to infect a host (Hogans & Trudeau, 1989).

Copepodid

The copepodid has a hydrodynamic body shape and is more elongated than the nauplius larvae (Fig. 1.4). The body is clearly segmented and consists of a fused head-chest part covered by a head-chest-shield which is called the cephalothorax and has thoracic leg bearing somites. Then there is a segment that represent the genital segment, and then the abdomen.

The cephalothorax is covered with a dorsal shield and a frontal plate in the front. Anterior and on the sides of cephalothorax, we find the first and second pair of antennas and on the ventral side is a mandible, two pairs of grasping appendages (second pair of antennas and maxilliped) and at least eight pairs of sensory setules. One of the pair with sensory setules marks the position of the eyes, that are situated inside the cephalothorax, under a transparent cuticle. The eyes have two lenses. The copepodid have two pairs of antennas for swimming, situated on the chest segments, and on the legs are pinnate setae to increase the resistance in the water, which gives a better swimming capacity (Piasecki, 1996). Older copepodids have developed the frontal filament in a membrane covered pocket in the cephalothorax (Piasecki & MacKinnon, 1993).

The copepodid is the infective stage and needs a host to continue its life cycle. It swims better than the nauplius-larvae and the swimming legs has an intense beating pattern to make an upward movement in the water column and sinks downwards when the legs are resting. This gives a characteristic irregular movement pattern.

The copepodid have two pairs of grasping appendages, second pair of antennas and the maxillipeds, which gives the copepodid a temporary attachment to the host. The frontal filament is being pushed forward from the pocket inside the cephalothorax and gives the lice a permanent attachment to the fish. The frontal filament will attach either to a fish shell or a fin ray, and will molt into the next stage, chalimus I (Piasecki & Mackinnon, 1995). The copepodid is positively phototactic and negatively rheotactic, which means that it swims against the water current (Piasecki & Mackinnon, 1995). The copepodid is also chemotactic and will swim towards a host if it is within 10 cm, regardless of a light source from another direction (Hogans & Trudeau, 1989).

Chalimus I-IV

Chalimus I has somewhat the same body shape as the copepodid, but is more elongated and wider (Piasecki, 1996) (Fig. 1.4). Its swimming legs are now reduced since it does not need to swim any further. The grasping appendages are similar to the ones on the copepodid (Hogans & Trudeau, 1989). The segmentation of the copepodid is gone and it is hard to tell if some of the thoracic somites are incorporated into the cephalothorax (Piasecki, 1996). The genital segment is clearly marked but not segmented (Hogans & Trudeau, 1989).

Chalimus II has a more distinct segmentation than chalimus I. The second somite is incorporated into the cephalothorax. The genital segment makes up a considerable part of the

body. Its frontal end is regularly triangular, with frontal plates that are partly separated from the back shield (Piasecki, 1996).The swimming legs are shorter and wider than the ones in chalimus I (Hogans & Trudeau, 1989).

Chalimus III is longer and wider than chalimus II and morphological, it resembles chalimus II, but with a clearer segmentation. The third leg-bearing segment is incorporated into the cephalothorax. The frontal end is regularly triangular, as the chalimus II, with defined frontal plates (Piasecki, 1996). The frontal plate has structures that will later become lunules, structures that works as a sucking device in the adult lice (Hogans & Trudeau, 1989). In this stage it is possible to separate the sexes from each other. The male abdomen is divided into two parts, while the female abdomen consists of only one segment. In addition, there are some differences on the second pair of antennas (Piasecki, 1996).

Chalimus IV is flatter than chalimus III and the back shield is parted into plates. The frontal end is triangular with a rounded front. It is now easier to morphological separate the sexes from each other, because they have different relative proportions and differences in the first and second pair of antennas and the maxillipeds. Both have a longer and slimmer genital segment (Piasecki, 1996). The segmentation of the body is mainly like the adult, the swimming legs are bigger than in chalimus III, and covered in pinnate setae. The female and male chalimus stay similar in structure through the last ecdysis (Hogans & Trudeau, 1989).

All chalimus larvae inherit the frontal filament from the previous stage and the length on this filament will determine the larvae's grazing area. Just before ecdysis there is an extension filament (extension lobe) visible under the old shell. When the lice go through its next ecdysis, this shell will break at the frontal end. The frontal filament is still stuck in the old shell. The elongated filament on the inside of the old shell is connected to the old filament and is hardened in contact with water. The frontal filament is therefore changed a bit for each stage and can contribute to stage determine the chalimus larvae (Piasecki & MacKinnon, 1993).

Adult

Young adult females have an elongated, rectangular genital segment, which grows significantly in width as the female grows older and becomes almost square (Fig. 1.4). The male genital segment is far smaller than the female genital segment, and has an elongated, oval shape. Young and older males have a similar morphology (Piasecki, 1996). Adult sea lice

have a characteristic light yellow-brown color along the edges and is darker in the middle (Heuch & Schram, 1999). The female is often seen with two long egg strings attached to the genital segment.

The cephalothorax is divided into plates and in the front, there are frontal plates with two lunules. All species in the genus *Caligus* have such lunules (Hogans & Trudeau, 1989). This is an important characteristic of *C. elongatus*, as salmon lice do not have lunules. Both the female and the male have two pairs of powerful grasping appendages (second pair of antennae and the maxillipeds) and four pairs of swimming legs. The swimming legs are situated further back (posterior) than the grasping appendages (Hogans & Trudeau, 1989). Young adults can still attach to the fish with the frontal filament and both females and males can retain the frontal filament throughout their life without it being attached to the fish (Hogans & Trudeau, 1989; Piasecki, 1996).

The lunules can be used to attach to the host temporarily or permanently at various stages of life (Kaji et al., 2012). The body of the lice works almost like a suction cup, the marginal membrane on the side of the cephalothorax, the frontal plates at the front and the third pair of legs at the back, provide a tight seal against the fish (Kabata, 1992; Pike & Wadsworth, 1999). Together with the grasping appendages, this gives the lice a good grip to the surface of the fish. The swimming legs enables the lice to move freely in the water or on the surface of the fish, and to move between hosts (Hogans & Trudeau, 1989).
Females and males become sexually mature at the same time, but males are more mobile than the female to search the fish for a female to mate with. They mate with young females that are still attached with the frontal filament (Piasecki & Mackinnon, 1995). A male louse is also capable of holding on to a female chalimus IV and wait until she is sexually mature (precopula). The females have a spermatophore that stores sperm from the male and gradually releases this to fertilize eggs that comes from the ovaries. Young females often already carry sperm from a male. The eggs mature in the genital segment before they are deposited into egg strings (Hogans & Trudeau, 1989). The egg strings that are produced have a light color. As the embryo develops, pigment is produced in the embryo and the egg strings develops a darker, brown color (Pike et al., 1993).

1.5 Host preference and genotypes

C. elongatus is a generalist and can infect more than 80 different fish species (Kabata, 1979). Different studies have shown that both cod (*Gadus morhua*), tub gurnard (*Chelidonichthys lucerna*), pollack (*Pollachius pollachius*), sea trout (*Salmo trutta*), herring (*Clupea harengus*), and saithe (*Pollachius virens)* are favorable hosts for *C. elongatus* (Boxshall, 1974; Heuch et al., 2007; Øines et al., 2006). Lice infestations occurs on both wild and farmed salmonids, but a study showed that arctic charr (*Salvelinus alpinus*) were more prone to be infected by *C. elongatus* than the Atlantic salmon (Mustafa et al., 2005).

C. elongatus is considered as one species with two genotypes, genotype 1 and 2 (Øines & Heuch, 2005). There are reports that show a higher abundance of genotype 1 on lumpfish and that genotype 2 found more on saithe. There is also a seasonal change of genotypes, as a study by Øines & Heuch (2007), discovered that genotype 1 was more represented in wild fish samples collected during spring and that genotype 2 gradually increased in samples collected during autumn.

1.6 Comparing the effects on the host with *L. salmonis* and *C. elongatus* infections

When the sea lice attach to their preferred host, the host will experience different negative effects. However, it is found that the damage comes from the lice feeding on the fish and not from the attachment or movement of the lice (Kabata & Hewitt, 1971). Infestations can be seen in increased jumping activity, irritated skin, reduced feeding activity, weight loss or mortality. Sea lice strip away epidermal tissue and areas of mucous, allowing secondary bacterial or viral infections to infect these areas (Wootten et al., 1982). The final cause of death of sea lice infected fish is often osmoregulatory failure (Hogans & Trudeau, 1989). The positioning of the two lice species are slightly different, and it is described that *C. elongatus* have a tendency to gather on the dorsal and lateral surfaces of the head and on the anterior portion of the abdomen between the opercula (Hogans & Trudeau, 1989). A study done by Treasurer and Bravo (2011) showed that adult *Caligus* species usually gather at the abdominal surface of the body, and adult *L. salmonis* are more seen on the back and on the head of young salmon. Chalimus of *C. elongatus* was found more on the fins.

1.7 Developmental time

Developmental time for crustaceans is strongly related to temperature, which means that sea lice have a shorter generation time at higher temperatures, and longer at colder temperatures (Costello, 2006). In a study conducted by Piasecki & Mackinnon (1995), they investigated the

life cycle of *C. elongatus* under laboratory conditions. The host fish they used were Arctic char (*Salvelinus alpinus*), and the development of the sea lice was studied at 10°C. They discovered that it takes around 8 days from the production of egg strings to hatching at 10°C. New eggs are ready inside the female's genital segment, and she produces new egg strings within a few hours after the first eggs have extruded from the egg strings, when the eggs hatch directly into nauplius I larvae. At 10°C the nauplius I go through ecdysis within 24 hours and becomes nauplius II. Nauplius II stage lasts approximately 67 hours at 10°C, before ecdysis to copepodid stage (Piasecki & Mackinnon, 1995). Copepodids only molt after they have established themselves on a host fish. The results from their study revealed that the *C. elongatus* had a generation time of 43.3 days (6.2 weeks) at 10°C. They recorded the first and last appearance of the different stages in the cohort, and this data showed big individual differences. They also observed their first chalimus 1 after only 2.9 days at 10°C.

1.8 Aims and hypothesis

Previous research conducted on sea lice is focused primarily on salmon lice. It would therefore be of great interest, for both research and commercial purposes, to further investigate the developmental stages of *C. elongatus* and how different temperature affect these sea lice. The paper of Piasecki & Mackinnon (1995) investigated the development of *C. elongatus* under one temperature (10 °C) and some of their results were very different to the development of salmon lice. A new experiment on *C. elongatus* should therefore examine the development over a broader variety of temperatures. The aim of the present study was to observe and record the parasitic, embryonic, and planktonic development of *C. elongatus* under four different temperatures: 6, 9, 12 and 15 °C. Part 1 included studying the infection success, the days post infection (dpi) to adult stage, 1st extruded egg strings and 2nd extruded egg strings. Part 2 included studying the hatching success and survival to copepodid, the days post hatching (dph) to the hatch of next egg batch, to nauplius II and to copepodid, and till death of copepodids.

The hypothesis was that temperature would affect the parasitic, embryonic, and planktonic development and size of *C. elongatus* significantly.

2 Material and Methods

2.1 Experimental Animals

2.1.1 Atlantic salmon

Atlantic salmon *Salmo salar* post smolts (Aquagen strain) from a single cohort were used for propagation of lice, with a mean weight of 477.5 g (±157.0 g) and mean fork length of 33.8 cm (± 3.8 cm). Fish were fed to satiation (Skretting Spirit S, pellet size 75 and 150) and kept in tanks with a continuous flow-through of filtered and UV-treated seawater (34.5 ppt) pumped from 90 m depth in the adjacent fjord. Fish were acclimated at least 14 days prior to the experiment. A total of 120 fish were distributed among 12 tanks, with 10 fish in each tank. All experiments were conducted in accordance with the Norwegian legislation for animal welfare at Matre Research Station of the Institute of Marine Research, Norway (Mattilsynet ethics approval ID #23820).

2.1.2 Sea lice

"Skottelus" (*Caligus elongatus*), hereafter referred to as sea lice, were used in this study. Lice were sourced from a variety of places; farm sites at Austevoll (IMR) and Prestholmane (Bremnes Seashore), and a production stock from University of Bergen. Numerous attempts at transporting lice proved unsuccessful in providing experimental infection. The lice were transported in plastic sealed containers that were stored in Styrofoam boxes with ice to keep the correct temperature during transportation. One lice cohort from a lab strain at UiB were successful at infection at Matre and became the source population that provided eggs for the experiment. Therefore, lice and egg strings were produced at the same facilities as the experiment were run in. The lice would then infect our experimental salmon and the lice unable to remain on their host were lost from the system.

2.2 Experimental design

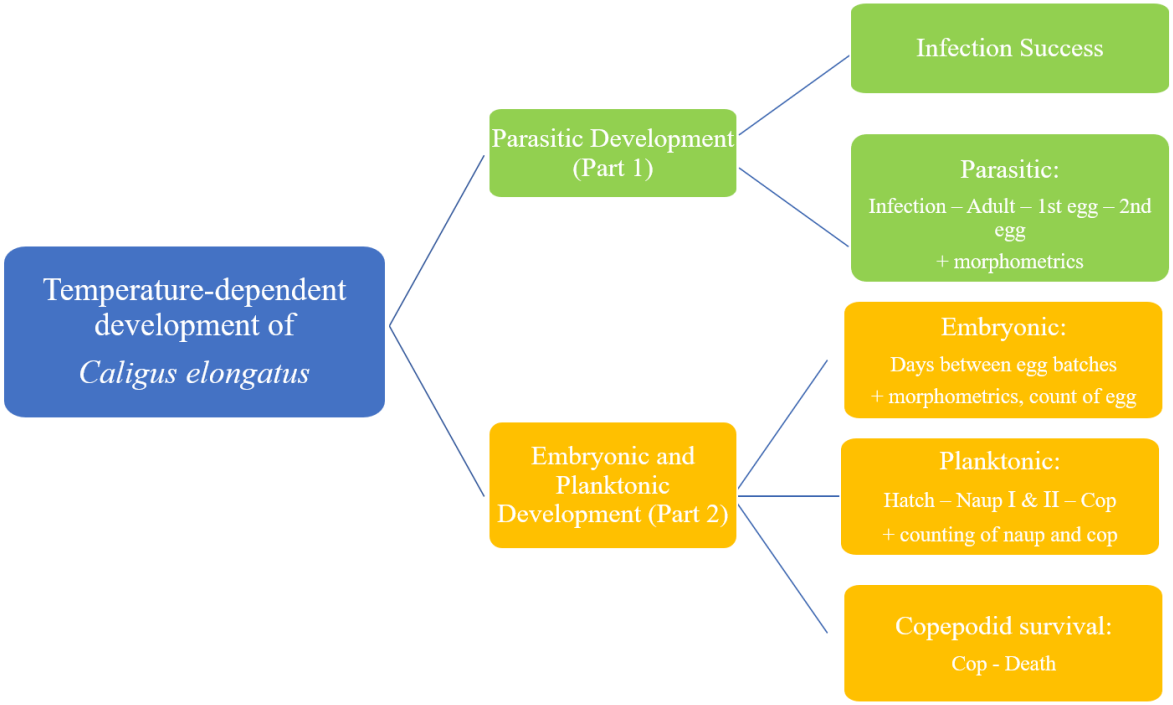

Fig. 2.2: Overview of the full experiment

The experiment consisted of two parts of measuring the development of *Caligus elongatus* at four different temperatures: 6, 9, 12 and 15 °C (Fig. 2.2). Part 1 included observing and recording the duration from infection to adult stage and the extrusion of its 1st and 2nd egg strings. Part 2 included recording the embryonic and planktonic development, to observe the development from egg to copepodid stage. Lastly, the copepodid survival would be observed and recorded. When referring to the different groups of lice on the different temperatures, the abbreviations T6 will be used for temperature group 6°C, T9 for temperature group 9°C, T12 for temperature group 12°C and T15 for temperature group 15°C.

2.2.1 Parasitic development (Part 1)

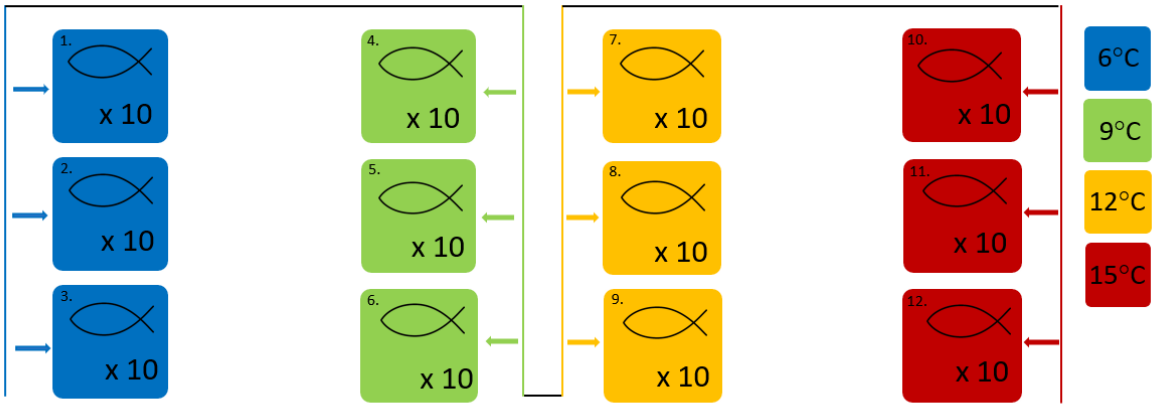

Fig. 2.2.1: Waterflow system. Each box is a tank with 10 fish inside. Each temperature group has 3 tanks each and which gets the same temperature from their respective header tank.

Part 1 of the experiment consisted of recording the stage and the abundance of lice over time. The development of their growth was measured under the temperatures 6, 9, 12 and 15 °C. Each temperature group consisted of three fish tanks containing 30 fish in total with 10 fish in each tank (0.9 x 0.9 x 0.4 m deep; volume ca. 0.32 m^3) (Fig. 2.2.1). Fish tanks were provided with continuous light and supplied with 34 ppt seawater at temperatures held at a stable 6, 9, 12 or 15 ± 0.1 °C. Temperatures were measured continuously using digital thermometers within the header tanks supplying the experimental tanks (1 header tank per 3 experimental tanks). These header tanks were temperature-adjusted manually; however, once set, did not deviate by more than 0.1 °C. Temperatures were monitored with a management system provided by Normatic (www.normatic.no) that sends an alarm when conditions outside the set thresholds are sensed.

Lice estimation

Prior to the infection, an estimate of the number of copepodids in a known volume of water was conducted. The copepodids were poured from the incubators into a large beaker with seawater at the same temperature as the incubators. The number of liters in the jug was noted. The water was agitated to create homogeneous distribution of larvae, then 20 mL aliquots were taken and transferred into a counting tray, where the number of copepodids were counted through the microscope and the abundance in the total volume was estimated. This was repeated 6-8 times, which gave an average over the total number of copepodids available for infection.

Infection Procedure

The experiment followed a standard infection procedure: tank water level was reduced to 1/3 of the normal volume and water inflow was adjusted to 12-15 l/min before copepodids were added. Tank outlets were blocked until normal tank levels had been reached (20-30 min), and thereafter normal water flow (25 l/min) was re-established. Oxygen levels were monitored to ensure saturation did not fall below 60%. Due to limited number of lice larvae available, not all temperatures were infected simultaneously. The first temperature groups to be infected were T9 and T12. When there were sufficient lice copepodids, both T6 and T15 were infected. T6 were infected with 50 copepodids/fish. T9 were infected with 60 copepodids/fish. T12 were infected with 41.6 copepodids/fish. T15 were infected with 50 copepodids/fish. The number of copepodids were slightly different due to the available copepodids at the time.

Sampling Procedure

For each temperature group, 8 samples were executed to record the stage and abundance of lice over time. Sampling times were calculated from a preliminary development model (Hamre, L. unpublished data). An overview of all sampling events and lice counts can be found in Table 6.1 in the Appendix. Sampling began when the lice were expected to be chalimus 4 stage, in order to avoid frequent handling of fish and the potential associated parasite loss. Low infection success was expected, and therefore we wanted to maximize the chance of observing adults and their egg string production.

For each sampling, the flow was stopped and the water level in the tank was lowered, and a small dose of metomidate hydrochloride (Aquacalm) [0.2g/100L]) was added to lightly sedate the fish. Two and two fish were carefully moved to a bath of full sedation (Aquacalm) [1g/100L], until all fish in the tank were processed. Lice assessments were conducted immediately once the fish were unconscious. Sampling events were planned on a rotation scheme so that each tank was only disturbed at every third sampling to minimize the stress applied to the fish and to lose less lice through too-frequent handling. The sampling included noting the number (abundance) and stage of each louse found on a fish. It was noted when the first and second egg strings were extruded, to the best estimate of the assessor. The length and weight of the fish were measured the first time a new tank was sampled. A photograph was taken at first appearance for male lice and every time for female lice appearance. This was for later re-confirmation of stages in cases of uncertainty and for the morphometry measurements of the lice.

2.2.2 Embryonic and Planktonic Development (Part 2)

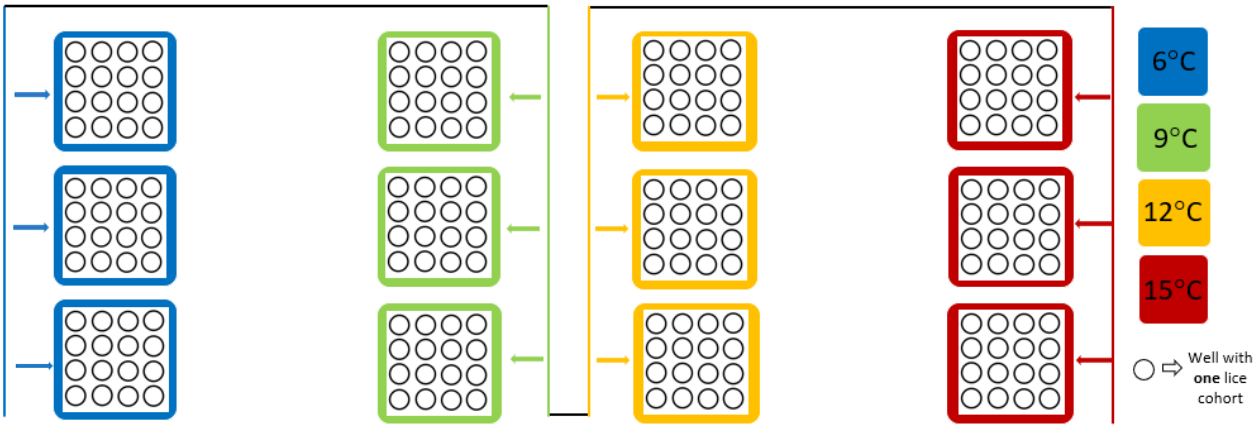

Fig. 2.2.2: Incubation and larval development system with the same temperatures as part 1. Each box contains 16 wells, where each well represents ***one*** *lice cohort from one mother louse.*

Part 2 of the experiment consisted of measuring the time between two egg batches, and to further follow the development of the lice from egg to copepodid stage. *C. elongatus* eggs are produced in batches and deposited in external egg strings. The frequency of egg batch production was measured by observing the time between hatching in two subsequent batches of eggs. To achieve this measurement, adult females needed to be individually identified.

Each female carrying egg strings was ID-marked and the egg strings were incubated in separate wells to keep the different cohorts separated from each other. In this way we were able to study the development of the different cohorts to hatch into nauplius I, and molt into nauplius II and copepodid stage. The incubation system consisted of shelves where individual boxes with 16 wells received a continuous filtered seawater supply (Fig. 2.2.2). Each well inside a box contained the eggs from one mother louse**.** The incubation system had the same temperatures as the first part of the experiment (6, 9, 12 and 15 °C), and the same water quality monitoring system. The temperatures were in addition monitored daily inside the incubators to ensure correct experimental temperatures (Appendix Table 6.2).

Embryonic development

Egg strings were carefully removed from adult females from the first part in the 6, 9, 12 and 15 °C groups and held individually in continuous flow incubators. To retrieve the eggstrings from females on the fish, the flow was turned off and the water level in the tank was lowered, and a small dose of the sedative Metomidate hydrochloride (Aquacalm) [0.2g/100L] was

added to calm the fish. Two and two fish were carefully moved to a bath of stronger sedative [1g/100L]. All female lice with egg strings were collected and the fish were put in a holding vessel. A photo was taken of each female with egg strings. The genital segments of the females were colored with marker pens (Fig. 2.4.1) in unique combinations (e.g. red:blue, red: black, blue: black), which gave a total of 16 different color combinations. The louse was dried gently with paper and thereafter with a breath of air, then marked with a color on the left and right genital segment, then air dried for the color to set. After marking the genital segment of the females, their egg strings were transferred to the incubator and the female lice were reattached to their hosts. When the next set of egg strings emerged, the lice were again removed from the fish using the same method, identified, and the new set of egg strings were incubated. The incubator wells were checked three times a day T9, T12 and T15 (09:00, 15:00, 21:00). T6 were checked two times a day (09:00 and 21:00), due to a slower development under this temperature. This was known from Hamre's preliminary developmental model and earlier studies on this louse. The duration of embryonic development was calculated from when the second lice cohort, from the same mother louse, hatched.

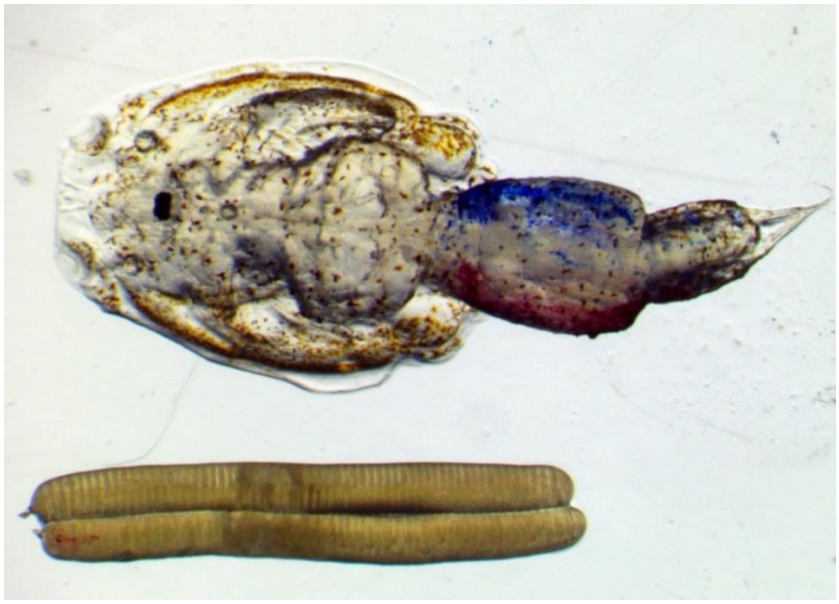

Fig. 2.4.1: ID-marked female with her egg strings at the bottom, with the color combination: Red-Blue

Planktonic development rate

Once the eggs hatched from the previous part, we continued to monitor them to determine planktonic development rate. The larvae go through two molts, from nauplius I to nauplius II and then to copepodid stage. The time between planktonic stages gave us the development rate of the planktonic stages of the offspring of the identified lice on the different temperatures 6, 9, 12 and 15 °C. After the eggs had hatched, the wells were checked once a day until they reached nauplius II. The wells were then only checked 1-2 days before it was expected that nauplius II would molt to copepodids, for less disturbance of the animals. The boxes containing wells were carefully removed from the incubation system to the lab for an inspection of the individuals inside the wells. Each well was sequentially emptied into a counting tray for the lice larvae to determine the stage and count the number of individuals under the microscope. Staging of the lice would be noted as a new stage (N2 or Cop) when more than 80% of the cohort in one well had molted into either nauplius II or copepodid stage.

Copepodid survival

In this part of the experiment, we wanted to study how long copepodids can survive without a host. Furthermore, we also wanted to see how temperature would affect the duration of survival. When the wells, containing the different lice cohorts, were completely molted to copepodid stage, they were transferred to another box and distributed among three wells (10 cops in each well). The sampling of the copepodid survival was done once a day around the same time each day. The box with copepodids was taken out of the incubation system and put on a light board, to illuminate the wells from below so that the copepodids could be better observed in the wells. The number of alive copepodids in each well were counted each day until 80 % of the population was dead. The wells were examined separately and in order. To decide whether the lice were dead or alive, we looked for movement of the copepodids. Only moving copepodids where counted as alive and approx. 15-20 seconds were spent studying each well.

2.3 Morphometric measurements

Fig. 2.3: Showing the parts of the lice that was measured in ImageJ. Dotted lines represent the cephalothorax, and the full line represents the total length of the lice. The eggs inside the egg strings are also seen as flat disks and were possible to count from picture.

From the images collected of the male and female adult lice, differences in body size between temperatures was determined by measuring multiple body parts. The morphometrics of the lice was measured using ImageJ (https://imagej.nih.gov/ij/) as a tool for exact size measurements of the pictures taken at the samplings.

Prior to each sampling in Part 1, an image was taken of a scale to calibrate ImageJ. When measuring the lice in ImageJ, the scale would provide the correct length (mm) of the different measured parts of the lice. In part 1, 79 female and 75 male lice were measured (total: 154), and in part 2, 118 female lice with egg strings were measured. The two parameters measured for all the lice was the total length (tot) and the cephalothorax (c) (Fig. 2.3). The lice were measured where the pigments were observed, and the outer membrane was not included. The

females with egg strings in part two also had their egg strings measured; left egg string (le) and right egg string (re). The eggs inside the egg strings used for further incubation and larval development was counted through the pictures using a marker function in ImageJ.

2.4 Data Analysis and Statistics

Analyses were conducted in R (The R Foundation, 2018). The parameters were compared among treatment temperatures using: ANOVA, GLMM, MANOVA, LDA, and Tukey's HSD test. Analyses were run in R by Samantha Bui, and results were interpreted by Gine Myhre.

Parasitic development (Part 1)

Each sampling consisted of noting the date/time and the abundance and stages of lice on the fish. The sampling day was noted as DPI (days post infection). No statistical analysis was conducted for this part since the results was best shown in a graph with the 1st observed, all adults and 1st observed adult with egg strings.

Embryonic & Planktonic development (Part 2)

Count of nauplii and copepodid larvae, hatching and survival success

Three GLMM models were generated to include a stepwise inclusion of Temperature and Sample, with LouseID as a random factor. The null model was that no factors would influence the outcome of the data. Model 1 had temperature as independent variable, model 2 had sample as independent variable and model 3 had both temperature and sample as independent variable and was therefore called the full model. The models were compared to the null model using an ANOVA; if significantly different from the null model, models were ranked and the one with the lowest AIC was selected. This model was run, and if applicable, a post-hoc Tukey's HSD test was run to determine the differences between the groups. This process was applied to the data for the number of eggs, nauplius II and copepodids. The same analysis was conducted for the hatching success (#N2/#Eggs) and survival success (#Cop/#Eggs).

Developmental Time

Each sampling consisted of noting the date and time when the different stages appeared. First it was noted when the egg strings hatched, then when nauplius II larvae were observed, and then when the copepodid stage was observed. When 80 % in one lice cohort was molted into a new stage, they would also be counted. Because development rate to nauplius II and copepodid would be intrinsically linked within cohorts, these two parameters were combined as the dependent variable in a MANOVA. Fixed factors included Temperature and LouseID.

The MANOVA was followed by univariate tests with a Bonferroni correction, to determine the differences between temperatures groups. In addition, a discriminant function analysis was applied to distinguish the nature of the difference between temperatures using their relationship with the development rates

Copepodid survival

The duration of survival for the copepodid stage was calculated from when 80% of the cohort were dead. These durations were analyzed using GLMM models with temperature as a factor, and Louse ID as a random factor. The null model predicted no factors would influence the data and model 1 had temperature as independent variable. The null and test model were compared using an ANOVA. If temperature significantly influenced survival duration, a post-hoc Tukey's HSD test was conducted to determine differences between groups.

Morphometric measurements

For adult male and females, only the total length and the cephalothorax length were analyzed. For the adult female lice, the total length was analyzed. The measurements were analyzed using GLMM models with temperature and sex as a factor and only temperature for the measurements of the adult female lice. An ANOVA was then conducted to compare the models to the null model. The output from the ANOVA would state whether the factors would influence the measured sized of lice, a post-hoc Tukey's HSD test would be conducted to determine difference between the temperature groups.

3 Results

The results will include graphs with corresponding tables and analysis from the observed stages of development of the different stages of the lice, and from the lice morphometry. Results from each part will be presented below, and a full table with all the developmental results is shown in Table 3 below.

Table 3: Containing all the developmental results from part 1 and 2 on the different temperatures; 6, 9, 12 and 15 °C.

	Measured development	Days post infection (I) / hatch (H)	Temperatures (°C)			
			6	9	12	15
Part 1	1st adult	DPI	33,6	22,8	15,8	11,3
	All Adult	DPI	43,7	26,6	17,7	12,8
	1st egg string	DPI	59	30,7	20,8	17,3
Part 2	Next hatch	DPH	12	6,8	4,6	3,2
	Nauplius II	DPH	1,9	1,5	1	0,7
	Copepodid	DPH	8	4,8	3,3	2,5
	Death	DPH	21,5	17,1	11,1	9,7

3.1 Parasitic development (Part 1)

3.1.1 Infection Success

The total amount of copepodids in a tank was calculated from the estimated number of copepodids per fish multiplied with the number of fish in the tank. The infection success was calculated with the number of lice observed in the first sampling, divided by the estimated copepodids in that tank. The infection success for both individual tanks and temperature group is listed below in Table 3.1.1. The individual infection success deviated greatly from the mean of each temperature group. Tank 13 had the lowest individual infection success with a percentage of 0.2 % and tank 6 had the highest infection success with a percentage of 16.7 %. Ranked from highest to lowest mean infection success is T9 with the highest infection success, then T6, then T12 and lastly T15 with the lowest infection success. The mean infection success for T9 is 6.4 times higher than the mean infection success for T15, 4.8 times higher than T12 and 3.9 times higher T6.

Table 3.1.1: Infection success (%) among the different temperature groups as well as the different tanks. 'Cop/fish' represents the estimated number of copepodids before infecting. The mean is calculated for each temperature group.

Temp (°C)	Infection date	Tank	Cop/fish	#Fish	#Lice 1st sampling	InfSucc	Mean InfSucc
6	01.08 20:00	1	50	10	17	3.4	
6	01.08 20:00	2	50	8	15	3.8	3.1
6	01.08 20:00	3	50	7	7	2.0	
9	01.08 20:00	6	60	10	100	16.7	
9	01.08 20:00	7	60	8	41	8.5	12.1
9	01.08 20:00	8	60	10	67	11.2	
12	08.08 19:00	9	42	10	7	1.7	
12	08.08 19:00	10	42	10	12	2.9	2.5
12	08.08 19:00	11	42	10	12	2.9	
15	08.10 14:00	13	50	10	1	0.2	
15	08.10 14:00	14	50	10	7	1.4	1.9
15	08.10 14:00	15	50	10	20	4.0	

3.1.2 Parasitic development rate

The results from this part gave us the days post infection to when the first, all adults and first adult with egg strings were observed for the temperatures: 6, 9, 12 and 15 °C (Fig. 3.1.2). Initially the goal was to observe both 1st and 2nd extruded egg strings but turned out to be difficult to distinguish between since there was no easy way of knowing which lice that had extruded its 1st and 2nd egg strings. The picture could help decide which egg strings was extruded but there were individual differences in the length of the egg strings. This is further explained in the discussion.

The lice in T6 had the slowest development rate with 43.7 days until all lice were adult. The fastest development rate was the lice in T15, with 12.8 days until all lice were adult.

When comparing all the temperature groups to T6 regarding the number of days to adult stage ('AllAdult'), T9 lice develop 39% faster (17.1 days), T12 lice develop 59% faster (26 days) and T15 lice develop 70% faster (30.9 days) than the T6 lice. In other words, T15 lice uses 30% of the time T6 lice use to reach adult stage, while T12 and T9 lice uses 40.5% and 60.9% of the time, respectively.

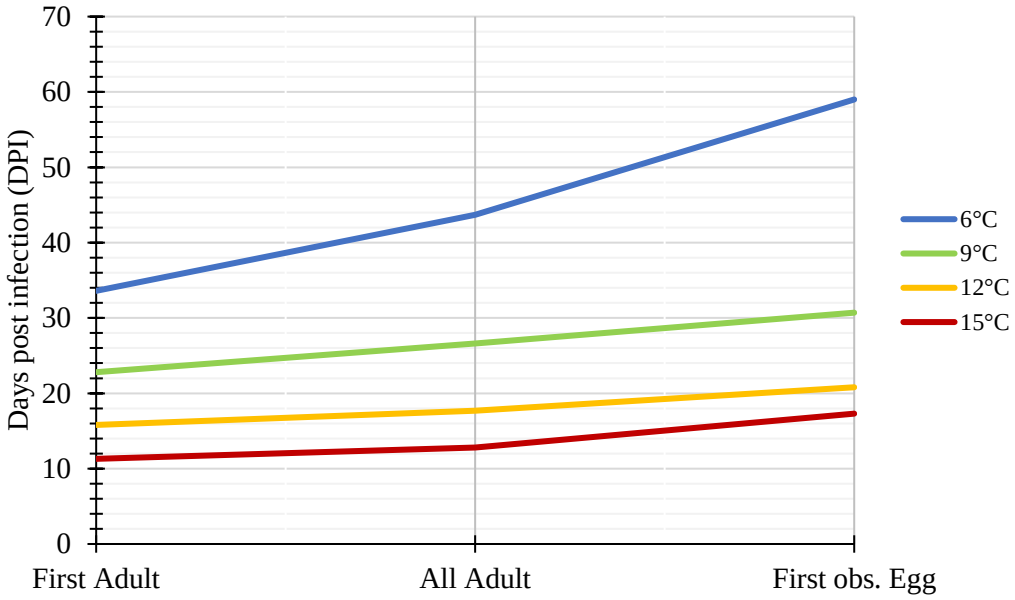

Fig. 3.1.2: Days post infection (DPI) until first observed adult ('First Adult') and all adults ('All adult'), and first observation of adults with egg strings ('First obs. Egg').

3.2 Embryonic and planktonic development (Part 2)

In this part, a total of 79 adult female lice were used to collect take egg strings and to further incubate them.

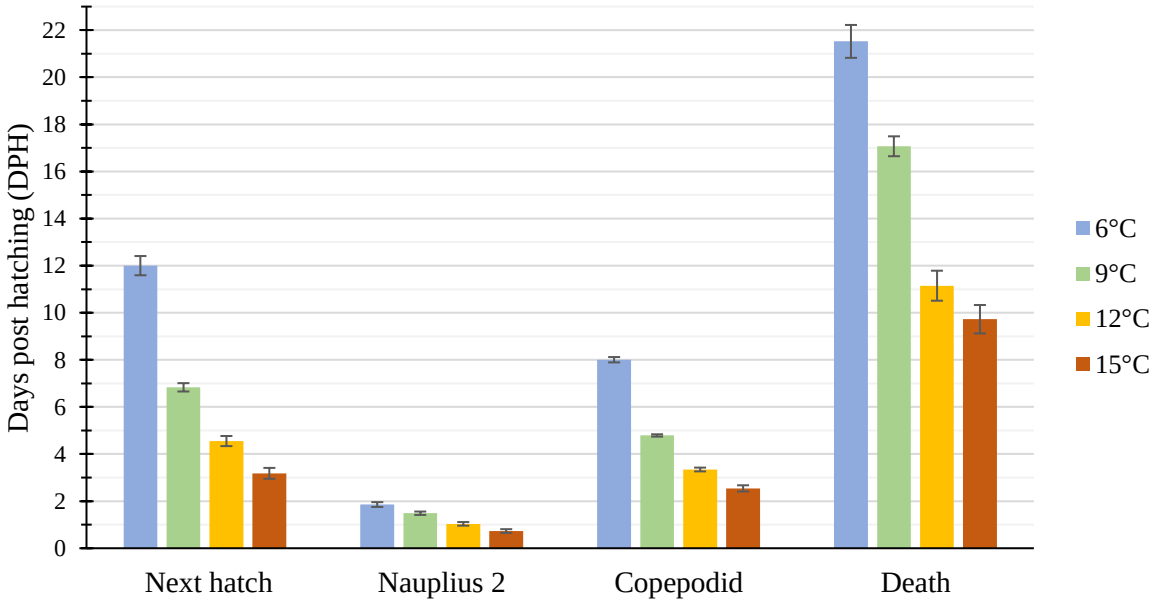

Fig. 3.2: Days post hatching to the different stages (Mean ± SE). 'Next hatch' is the embryonic development with the days between hatching of two subsequent egg strings. 'Nauplius II' is the DPH to when 80% of one lice cohort had molted into nauplius II. 'Copepodid' is the DPH to when 80% of one lice cohort had molted into copepodid stage. 'Death' is the DPH to when 80% of one lice cohort was dead.

3.2.1 Embryonic development rate

The number of lice used in this part varied among the different temperature groups. This is a result of how many lice available at the time, which also traces back to the different infection success. The recapturing of the ID-marked females also varied and will be discussed in the next chapter. The number of ID marked lice and recaptured lice in the different temperature groups are listed below in table 3.2.1a.

Table 3.2.1a: Number of ID-marked adult female lice and recovered lice

Temp (°C)	#Lice ID marked	#Lice recovered	% recovered
6	10	6	60.0
9	46	21	45.7
12	17	4	23.5
15	6	3	50.0

The embryonic development rate was therefore based on very few individuals for all the temperature groups except for T9. The days between hatching of two subsequent egg batches is listed in table 3.2.1b. and not analyzed due to a small sample size. From the results we can still see that T6 use the most days to develop new egg strings (12 days), then comes T9 with 6.8 days, T12 with 4.6 days and lastly T15 that use 3.2 days which is 73.3% faster than T6 (Fig 3.2, table 3.2.1b).

Table 3.2.1b: Embryonic development rate with days between two subsequent hatchings from the same mother louse. x(y, n=z): x=mean, y=SE, standard error, z=number of individuals

Temp (°C)	Days between egg batches
6	12.0 (0.4, n=6)
9	6.8 (0.2, n=21)
12	4.6 (0.2, n=4)
15	3.2 (0.2, n=3)

3.2.2 Planktonic development

In this subsection, counted individuals, hatching success and developmental time will be presented and described.

Count of eggs, nauplii and copepodids

The number of eggs, nauplii and copepodids from the temperature groups are listed in table 3.2.2a. T9 clearly differs from the other temperature groups, with 30% more counted eggs, 37% more counted nauplii and 38% more counted copepodids.

Table 3.2.2a: Table represents the eggs counted from pictures of the egg strings (#Eggs), and the counting of nauplius II larvae (#N2) and copepodids (#Cop) through microscope. x(y, n=z), x=mean, y=SE, standard error, z=number of lice cohort

Temp (°C)	#Eggs	#N2	#Cop
6	83.6 (9.3, n=17)	52.6 (9.3, n=17)	43.2 (7.7, n=16)
9	123.6 (4.0, n=67)	83.4 (4.4, n=75)	62.7 (3.5, n=73)
12	97.8 (6.2, n=24)	57.8 (5.3, n=21)	38.8 (4.6, n=20)
15	79.3 (4.7, n=9)	46.8 (6.1, n=9)	35.1 (6.0, n=8)

The model including only temperature was selected for in all three dependent variables (model selection results not shown), indicating that temperature was the only significant factor influencing the number of counted eggs, nauplius larvae and copepodids (Table 3.2.2b). A post-hoc test was conducted to compare the means of the different temperature groups, and the results showed that only T9 was different to all the other groups for the three individual models (for eggs, nauplii and copepodids).

Table 3.2.2b: Results of the selected model, from the ANOVA comparing the three models to the null model for the number of eggs, nauplius II and copepodids. The Chi-squared value (χ2) and p-value for the significant model are reported. The two last columns represent the results from the post-hoc test with the significant p-values in bold.

Description	Factor in selected model	AIC value	χ2	Chi df	*p*-value	Post-hoc group	*p*-value
#Eggs	Temperature	1090.5	21.4988	2	<0.001	9 - 6	0.002
						12 - 6	0.936
						15 - 6	0.880
						12 - 9	0.005
						15 - 9	0.001
						15 - 12	0.577
#N2	Temperature	1116.9	21.3148	2	<0.001	9 - 6	0.012
						12 - 6	0.998
						15 - 6	0.826
						12 -9	0.008
						15 - 9	0.003
						15 - 12	0.723
#Cop	Temperature	1043.0	16.3701	2	0.0003	9 - 6	0.109
						12 - 6	0.886
						15 - 6	0.823
						12 -9	0.003
						15 - 9	0.035
						15 - 12	0.990

Hatching success and survival of larvae

The number of eggs, nauplius II larvae and copepodids gave us the hatching success and the survival of larvae (Table 3.2.2c). The hatching success showed how many off the counted eggs inside the egg strings that hatched and survived to nauplius II. The percentage of the individuals that did hatch was less than 60% for T6, T12 and T15 and more than 70% for T9. The percentage of individuals that survived to copepodid stage was around 40% for T6, T12 and T15 and more than 50% for T9. Nevertheless, the statistical analysis showed that no models were different to the null model ($p > 0.05$), which indicates that neither temperature or sample affected the hatching success and the survival of larvae (Table 3.2.2c).

Table 3.2.2c: 'Hs Naup' is the relative hatching success for nauplius larvae compared to the number of eggs, and 'Hs Cop' is the relative survival success from egg to copepodids. x(y, n=z), x=mean, y=SE, standard error, z=number of individuals

Temp (°C)	Hs Naup	Hs Cop
6	0.57 (0.06, n=17)	0.42 (0.05, n=17)
9	0.73 (0.03, n=46)	0.52 (0.04, n=46)
12	0.58 (0.04, n=45)	0.39 (0.03, n=45)
15	0.59 (0.07, n=9)	0.40 (0.08, n=9)

Development from hatching to nauplius II and copepodid stage

The planktonic development rate is listed in table 3.2.2c below and shown in fig. 3.2. This development displays the mean days the lice used from hatching to nauplius II (DaysToN2) and to copepodid stage or duration of N1+N2 (DaysToCop) at the temperatures 6, 9, 12 and 15°C. The results from DaysToN2 showed that T6 developed slowest into nauplius II with a mean of 1.9 days (45.6 hours) and T15 developed fastest with a mean of 0.7 days (16.8 hours). The difference in developmental days between the lowest and the highest temperature is 1.2 days (28.8 hours). In other words, T15 uses 36.84 % of the time that T6 uses to become nauplius II. The results of DaysToCop also showed that T6 had the slowest development rate into copepodid with a mean of 8.0 days (192 hours) and that T15 had the fastest development rate with a mean of 2.5 days (60 hours), which resembles a difference of 5.5 days (132 hours). In other words, T15 uses 31.25 % of the time that T6 uses to become copepodid.

Table 3.2.2d: Planktonic development rate with the days post hatching (DPH) to nauplius II ('DaysToN2') and the DPH to copepodid stage ('DaysToCop'). x(y, n=z), x=mean, y=SE, standard error, z=number of individuals

Temp (°C)	DaysToN2	DaysToCop
6	1.9 (0.1, n=17)	8.0 (0.1, n=16)
9	1.5 (0.1, n=77)	4.8 (0.1, n=74)
12	1.0 (0.1, n=21)	3.3 (0.1, n=20)
15	0.7 (0.1, n=9)	2.5 (0.1, n=8)

A MANOVA tested the effect of temperature, LouseID on days to both N2 and Cop, which showed that temperature significantly affected the rate of development to these (Pillai's Trace V = 1.05, F3,28 = 10.3, P < 0.001). The following linear discriminant analysis (LDA) revealed a linear discriminant function which explains 99.8 % of the variance. Its coefficients highlight the large effect of temperature on DaysToCop (b = - 2.30) and a lower contribution of temperature in influencing DaysToN2 (b = - 0.72).

The post-hoc linear model conducted for DaysToN2 showed that the means for T6, T9 and T12 were significantly different to each other. T15 was not different to T12, which implies that the mean for DaysToN2 of T12 and T15 were very similar ($p > 0.16$). The result from the post-hoc Tukey HSD analysis is presented in a graph below (Fig. 3.2.2).

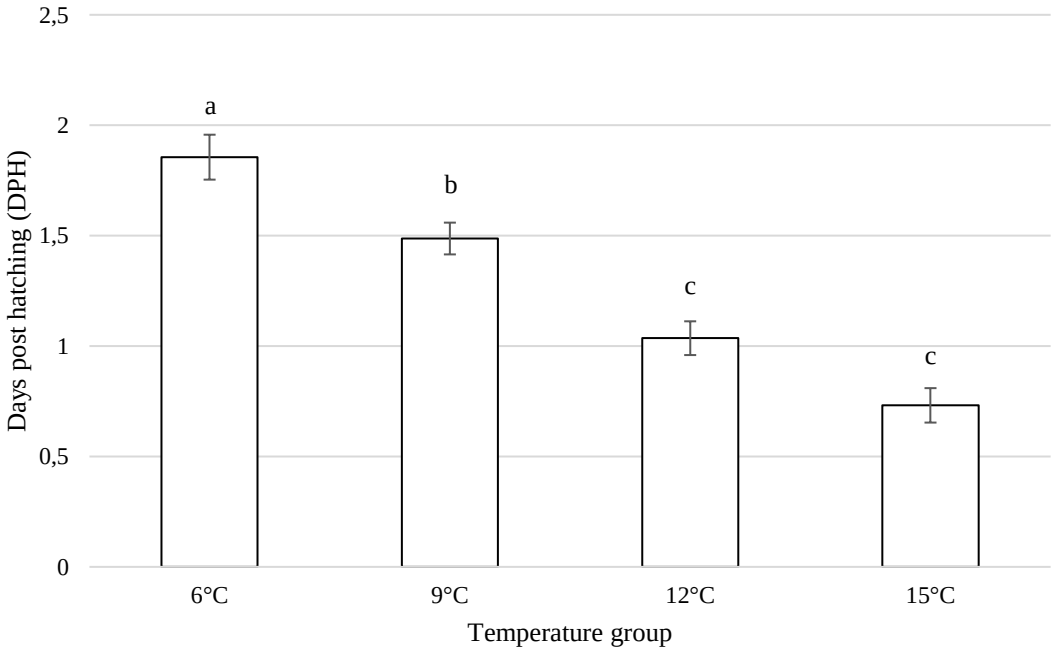

Fig. 3.2.2: Days post hatching to nauplius II, with different temperature (Mean ± SE). Post hoc differences at P < 0.05 are indicated by letters above the bars.

The post-hoc linear model conducted for DaysToCop showed that the means for all temperature groups were significantly different to each other. The results from both DaysToN2 and DaysToCop analysis are presented in a table below (Table 3.2.2).

Table 3.2.2e: Table represents the results from the statistical analysis

Description	Model	Coefficient	t-value	p-value
DaysToN2	Intercept	1.93	20.66	<0.001
	Temp9	- 0.54	- 5.27	<0.001
	Temp12	- 0.90	- 7.23	<0.001
	Temp15	- 1.20	- 7.87	<0.001
	Post-hoc group			
	9 - 6			<0.001
	12 - 6			<0.001
	15 - 6			<0.001
	12 - 9			0.001
	15 - 9			<0.001
	15 - 12			0.157
DaysToCop	Intercept	8.00	73.02	<0.001
	Temp9	- 3.24	- 26.73	<0.001
	Temp12	- 4.65	-32.12	<0.001
	Temp15	- 5.46	- 29.39	<0.001
	Post-hoc group			
	9 - 6			<0.001
	12 - 6			<0.001
	15 - 6			<0.001
	12 - 9			<0.001
	15 - 9			<0.001
	15 - 12			<0.001

3.2.3 Copepodid survival

The duration copepodid survival is listed in table 3.2.3a below and show the mean days from the time they were observed at the copepodid stage to death, and it shows the total number of days from hatching to death of the different lice cohorts. The results revealed (CopToDeath) that T6 survived the longest (13.5 days) compared to the other temperatures groups. T15 survived the shortest (7.2 days), which was close to half of the days (- 46.7%) of T6. The mean days of survival for T6 and T9 were close and only differs with 1 day. The mean days for T12 and T15 are also close and only differs with 0.6 days (14.4 hours), which is statistically proven in the post-hoc analysis (Table 3.2.3b).

Table 3.2.3a: Duration of copepodid survival. 'CopToDeath' is the days from copepodid stage to death (80 % of lice cohort). 'HatchToDeath' is the total days from hatching to death (80 % of lice cohort) x(y, n=z), x=mean, y=SE, standard error, z=number of individuals

Temp (°C)	CopToDeath	HatchToDeath
6	13.5 (0.7, n=13)	21,5 (0.7, n=13)
9	12.5 (0.4, n=19)	17.1 (0.4, n=19)
12	7.8 (0.6, n=16)	11.1 (0.6, n=16)
15	7.2 (0.5, n=7)	9.7 (0.6, n=7)

The model comparisons showed that the results were significantly different from the null model (Table 3.2.3b), indicating that temperature influenced the duration of the copepodid stage. The post-hoc Tukey HSD test showed that T9 was not different to T6 ($p > 0.613$), and T15 is not different to T12 ($p > 0.927$). All the other temperature groups were significantly different from each other. The result from the post-hoc Tukey HSD is presented in a graph below (Fig. 3.2.3).

Table 3.2.3b: Results from the ANOVA comparing model 1 to the null model for the Copepodid Survival. The Chi-squared value ($\chi 2$) and p-value for the significant model is reported. The two last columns represent the results from the post-hoc test with its p-values for the different groups.

Description	Factor in selected model	AIC value	$\chi 2$	Chi df	*p*	Post-hoc group	*p*
CopToDeath	Temperature	249.74	46.042	3	<0.001	9 - 6	0.613
						12 - 6	<0.001
						15 - 6	<0.001
						12 - 9	<0.001
						15 - 9	<0.001
						15 - 12	0.927

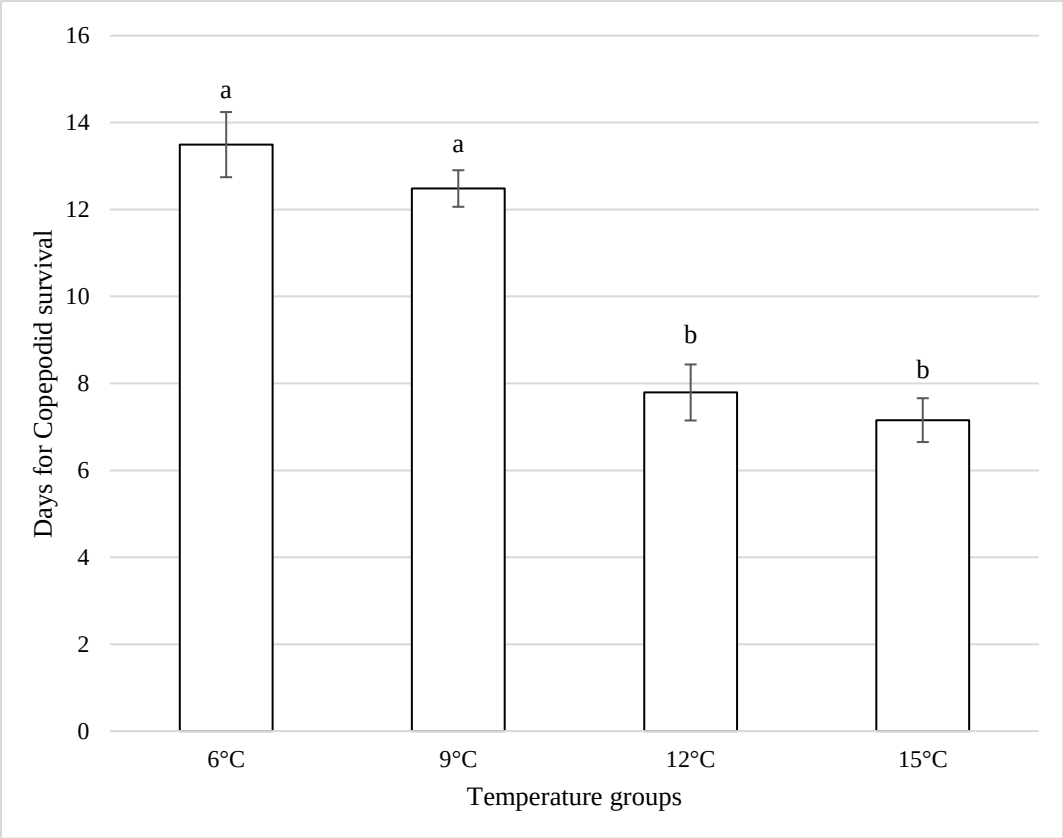

Fig. 3.2.3: The duration of days for copepodid survival for the different temperature groups (Mean ± SE). Post hoc differences at P < 0.05 are indicated by letters above the bars

3.3 Morphometric measurements

Measurements of adult female and male lice (Part 1)

The morphometric measurements for adult female and male lice are shown below in Fig. 3.3.1.

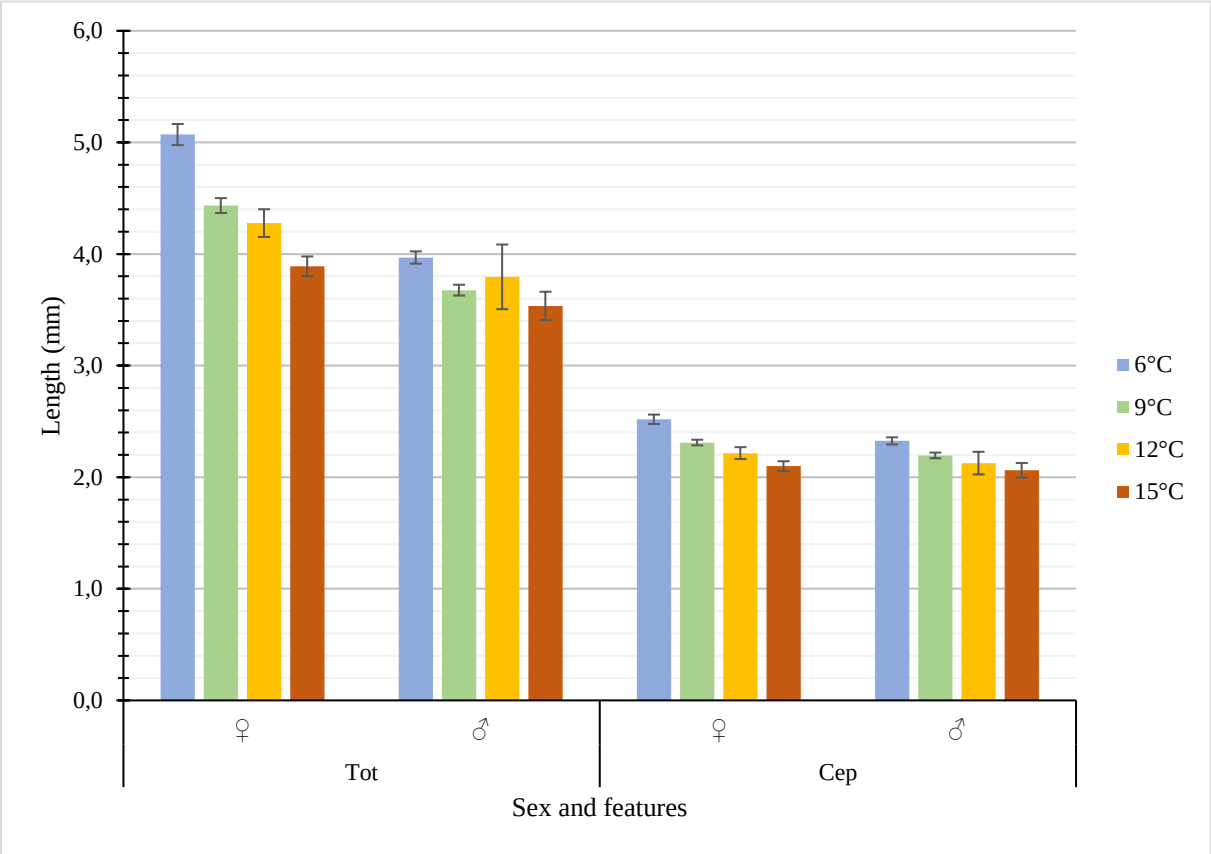

Fig. 3.3.1: Morphometric measurements of adult lice (Mean ± SE). 'Tot' represents the total length of the female and male lice, and 'Cep' represents the cephalothorax length of the female and male lice. ♀ = Female, ♂ = Male

The overall result from this part shows that T6 adult female lice have both the longest total length and cephalothorax length. The length of the lice in T9 and T12 are similar. The mean total length for T12 male lice is longer than the mean total length of the male lice at T9, but only four males were measured in T12 which could affect the results to some degree.

The total length of lice in T6 shows that females are 21.6 % longer than the male lice. T9 females are 15.9 % longer, T12 females are 11.6 % longer and T15 females are 10.3 % longer than the male lice. For both females and males, T6 are 13.9 % longer than T15 lice. The length of the cephalothorax didn't vary just as much between female and males, but the cephalothorax length of T6 females are 16 % longer than the female lice in T15.

The statistical analysis showed that cephalothorax and total length was significantly influenced by both factors: temperature and sex (Table 3.3.2).

Table 3.3.2: Results from the ANOVA from the morphometric measurements of adult males and females.

Description	**Factor in selected model**	**F-value**	**Df**	**Residuals**	***p***	**Post-hoc group**	***p***
Cep length	Temperature	15.34	3	149	<0.001	9 - 6	0.001
						12 - 6	0.003
						15 - 6	<0.001
						12 - 9	0.560
						15 - 9	<0.001
						15 - 12	0.324
	Sex	18.95	1	149	<0.001		
Tot length	Temperature	nov.23	3	149	<0.001	9 - 6	0.046
						12 - 6	0.520
						15 - 6	<0.001
						12 - 9	0.987
						15 - 9	0.048
						15 - 12	0.162
	Sex	140.29	1	149	<0.001		

The post-hoc Tukey HSD was conducted for the factor Temperature for both the cephalothorax and the total length of the lice. The test outputs showed that the cephalothorax length for T12 wasn't significantly different from the T9 ($p > 0.560$) and T15 wasn't different from T12 ($p > 0.324$). The other temperature groups were significantly different from each other (all comparisons, $p < 0.05$). For the total length, the post-hoc outputs showed that the only temperature group that were significantly different were T15 and T6 ($p < 0.001$).

Measurements of adult female lice with egg strings (Part 2)

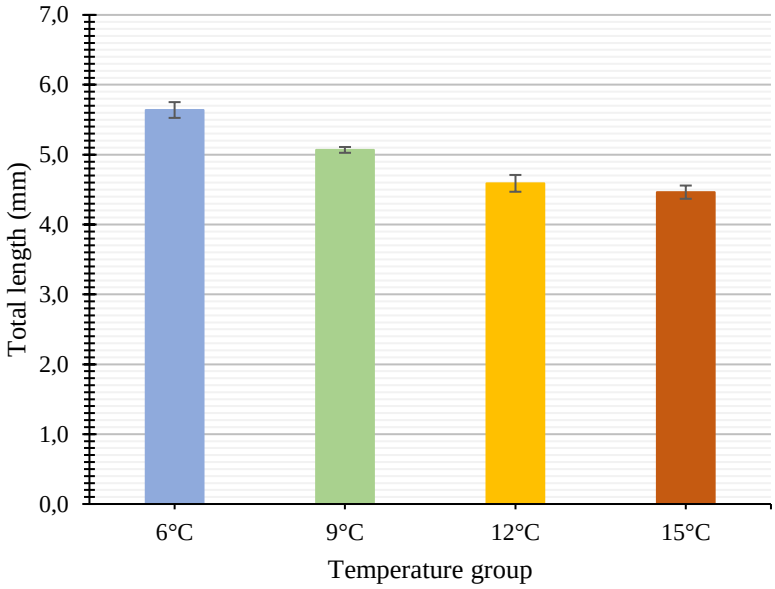

Fig. 3.3.3: Morphometric measurements of adult female lice with egg strings (Mean ± SE). Above the bars is the number of individuals and the mean.

The mean total length of the adult females with egg strings was longest in T6, then T9, T12 and T15 with the shortest total length. The length of T6 lice were 19.6% (diff: 1.1 mm) longer than T15. The groups with the closest mean were T12 and T15 lice with a difference by 0.1 mm, which is also shown in the analysis. The post-hoc Tukey HSD showed that T15 wasn't different from T12 ($p > 0.869$). The other temperature groups were significantly different from each other (all comparisons, $p < 0.05$). This is presented in table 3.3.4 below.

Table 3.3.4: Results from the ANOVA and post-hoc analyses of the Morphometric measurements part 2

Description	Factor in selected model	F-value	Df	Residuals	*p*	Post-hoc group	*p*
Tot length	Temperature	26.27	3	115	<0.001	9 - 6	<0.001
						12 - 6	<0.001
						15 - 6	<0.001
						12 - 9	<0.001
						15 - 9	<0.001
						15 - 12	0.869

4 Discussion

The developmental of the sea lice, *Caligus elongatus*, was studied under four different seawater temperatures (6, 9, 12 and 15°C) to study the effect of temperature on the rate of parasitic, embryonic, and planktonic development, and additionally morphometric measurements were conducted. The main discovery was that both the development time and the measured size of *C. elongatus* was strongly influenced by water temperatures.

4.1 Parasitic development (Part 1)

4.1.1 Infection Success

Prior to the infection of the host fish, we needed enough lice to fully infect the four temperature groups. The infection success in the individual tanks varied from the means within the temperature groups. The preparation of aliquots containing estimated numbers of copepodids could be a source of the variation between the individual tanks within the temperature groups. In this study, the estimated number of copepodids for each temperature group were slightly different but T9 (9°C) had the highest estimated copepodids per fish (60 cops/fish). T9 also had the highest infection success, which could be a result of the high estimated copepodids prior to the infection. The other temperature groups had rather low infection success ranging from 3.1 to 1.9 %. In our experiment T9 was the best infected groups and should be further investigated whether this is the optimal temperature for *C. elongatus*.

The lice infecting the 15°C fish had the lowest infection success, which is rather the opposite of the infection success for salmon lice, where they found an increase in the success with warmer temperatures (Dalvin et al., 2020). Prior to the infection in our experiment, the eggs were incubated at 11°C and had an acclimation time of 4 hours before infecting the 15°C fish. Each hour, the temperature of the water containing the lice, was increased one degree. The rather quick increase of temperature could have made the lice less fit for infecting the host fish. However, salmon lice have exhibited good infection success at cold temperatures even though produced at higher temperatures Dalvin et al. (2020).

The infection success for *L. salmonis* was calculated in a trial by Dalvin et al. (2020), where the estimated number of copepodids per fish was 40. The infection procedure was executed in the same manner as our experiment, and the tanks used were also the same. The major difference in procedure for infecting the host fish, was that they reduced the water inflow to 6

L/min, and we had a flow of 12-15 L/min. Their infection procedure was performed under 8 different temperatures ranging from 2 to 10°C, where the infection success was highest at the warmer temperatures. Comparing the infection success for both experiments, their infection success was 20-50 %, whereas our infection success varied between 2-12 %, with the highest infection success at 9°C. This shows a much lower infection success for *C. elongatus*, with 73 % lower infection success than the infection success for *L. salmonis* at 9°C.

Another difference separating the experiments was the time for first sampling and further calculating the infection success, first sampling for our experiment was when the lice were expected to be chalimus 3-4 (10-34 dpi), and their first counting was at late copepodid stage (4-11 dpi). However, this would not influence the infection success greatly since the lice are sessile from copepodid to chalimus 4 stage and lice would not fall off in this period.

4.1.2 Parasitic development rate

The developmental data by Piasecki & Mackinnon (1995) showed that the first appearance of adult *C. elongatus* is observed after 21.8 days at 10°C, while our data shows first observation of adult after 22.8 days at 9°C. Compared to *L. salmonis* (Hamre et al., 2019), our lice developed slightly faster into adult stage. The study by Hamre et al. (2019) used *L. salmonis*, whereby the development rate was recorded for both male and female lice at 8 different temperatures, and 4 of the same temperatures as our study. Hence, the data can be compared for the development of salmon lice to the development of *C. elongatus* at the temperatures of 6, 9, 12 and 15°C. This demonstrated that for all the temperatures *C. elongatus* uses approx. 61 % of the time the female salmon lice uses to reach adult stage, and approx. 77 % (72.2 – 80 %) of the time the male salmon lice uses to reach adult stage.

For the parasitic development, the goal was to record both 1st and 2nd extruded egg strings but turned out to difficult to distinguish between them. The pictures taken at the samplings would be helping with deciding whether the egg strings were the 1st or the 2nd pair. However, sometimes the samplings would occur in the period where the lice were in the middle of extruding her egg strings, which could be mistaken for a 1st egg string set. The lice with 1st egg strings would hatch in between the samplings and would therefore be stated as adult female lice without eggstrings. Fig. 4.1.2 is an example of the issue for this part. The two lice in the picture are carrying egg strings, where the left louse has extruded the 1st egg strings, while the right louse has extruded the 2nd egg strings. A further issue here is that the left louse was sampled approximately 8 days after the right louse.

This could of course trace to very individual differences in development time. (Hamre et al., 2019) – a previous study has argued that *C. elongatus* developed in a pattern much less synchronous than *L. salmonis* (Piasecki & Mackinnon, 1995) . Even so, the uncertainty of which egg string number was first observed in this study is too high to draw conclusions of development time until first or second egg string, and further research with more detailed observations during this period is required to further investigate this. As such, we negated this uncertainty by combining all observations of adult females with egg strings into one category called "1st observed adult with egg strings", which made the results more assertive.

Fig. 4.1.2: Adult female lice with egg strings under 6 degrees. Left louse (76 dpi) is likely to have her 1st extruded egg strings, and the right louse (68 dpi) is likely to have her 2nd set of extruded egg strings.

4.2 Embryonic and Planktonic development (Part 2)

4.2.1 Embryonic development

The results from this part were not analyzed due to a low number of individuals to calculate the duration of the embryonic development. T9 was the only temperature group with enough datapoints, but due to a low number of datapoints in T6, T12 and T15 it was not possible to compare the different embryonic development between the temperature groups statistically.

Qualitatively, the embryonic development (Days between egg batches) was highly influence by temperature. The embryonic development was faster with warmer temperatures, and slower with colder temperatures.

The embryonic development of *C. elongatus* compared to the development for salmon lice was slightly faster, with the same pattern as for their parasitic development (L. Hamre et al., 2019). For all the temperature groups, *C. elongatus* uses 63-70 % of the time salmon lice uses from one egg batch hatching to the next egg batch hatching.

A parameter that may have influenced the accuracy of the data was the actual temperature in the incubators, which was manually measured. The mean measured temperature for T6 was 6.2°C, and for T9 it was 9.6°C (Appendix Table 6.2). The two other temperatures were measured to be holding the correct temperatures of 12 and 15°C.

The procedure of ID-marking the lice could also be a source of error in this experiment. After the lice were colored (for individual ID), the lice would be attached to their host fish for some days before their next pair of egg strings to extrude. The ID-marked females would then be recaptured for further incubation of the next set of egg strings. For most of the lice, the color was only somewhat visible and for others, the color would be completely gone and the identifying of the lice through its color combination would not be achievable. It was important that the lice were somewhat dry before applying the color onto the lice, which could have been a reason that a great amount of the recaptured lice were unidentifiable. Another reason for the low recapturing of females could be that the full procedure of the ID-marking was too rough on the lice and that they did not survive until the next sampling of the next set of egg strings (recapturing). The lice that died or left the host fish would be lost from the system as there were no filter on the tank outlets.

4.2.2 Planktonic development

Counting of eggs, nauplii and copepodids

T9 differed from the other temperature groups in terms of larval production, with the highest mean number of both counted eggs, nauplii and copepodids. This could be a result of the higher number of lice cohorts counted for T9 compared to the other temperature groups. Even so, if T9 had a lower number of counted lice cohorts (e.g. 20), the mean would still be higher than the mean for the other temperature groups (data not shown). This can indicate that T9 lice were a more fit population or that 9°C is a more preferred temperature for the lice rather than temperature 6, 12 and 15°C. A temperature of 9°C is described to be the optimal

temperature for salmon lice (Tully, 1989) but is not known whether this is true for *C. elongatus*.

The counting of the individuals of eggs, nauplii and copepodids were executed manually through pictures (eggs) and microscope (nauplii and copepodids) (Fig. 4.2.2). The pictures of the lice with egg strings varied in how easy they were to count, and the nauplii and copepodids were counted in a tray but the individuals were moving quite a lot. Both counting methods could have human error, which could have affected the data for this part.

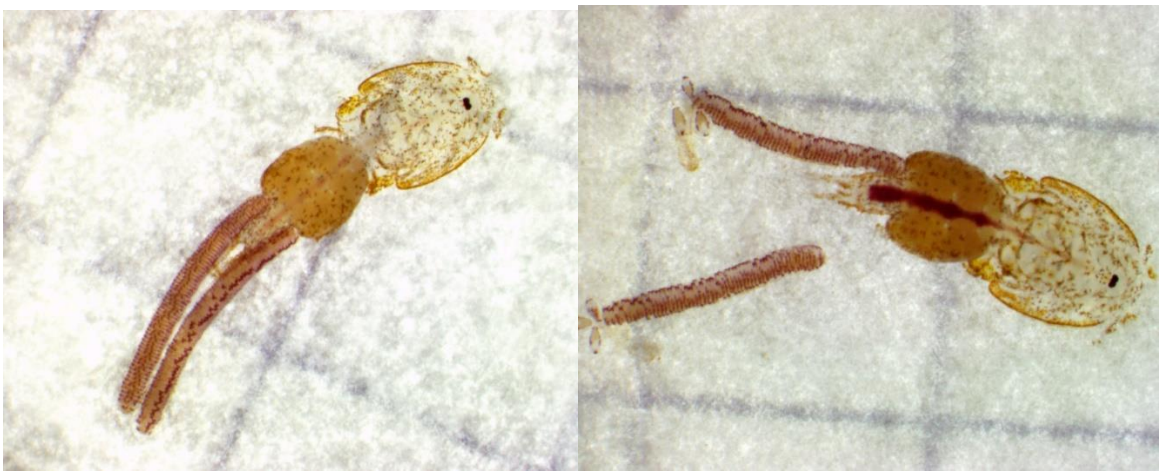

Fig. 4.2.2: Pictures of adult female lice with egg strings. Left picture: the eggs are observable inside the egg strings. Right picture: showing already hatched eggs into nauplii 1 and is observable at the end of the egg strings

Brooker et al. (2018) could display the relation between number of eggs per egg string for salmon lice at different water temperatures. This study showed that the egg strings produced at colder temperatures contained more eggs than the egg strings produced at warmer temperatures. Compared to our study, the mean counted eggs for the different temperature did not show this exact pattern, though this could be due to different number of counted individuals for each temperature. The highest number of eggs (123 eggs) were found in the lice produced at 9°C and the lowest number of eggs (79 eggs) were found at 15°C, which was the warmest experimental temperature.

Hatching success and survival to copepodid stage

Hatching success did not vary significantly among the temperature groups in this study. On the other hand, the opposite observation was recorded for salmon lice by Samsing et al. (2016), where their hatching success was strongly influenced by the different water temperatures. The hatching success at 15°C was 100 % for salmon lice and in our experiment

with *C. elongatus*, only 60 % of the eggs hatched. Other temperatures are not directly comparable to our experiment as they differed between the studies.

The hatching success for *C. elongatus* was similar for all the temperature groups (60-70 %) and the survival from egg to copepodid stage was around 40-50 %. Johnson & Albright (1991) conducted a study on the development of salmon lice, where reported that the proportion of nauplii that survived to develop to copepodids was around 50 % at 10°C. This is observed frequently in nature and is called r-selection where these species do not invest many resources into one offspring (K-selected species) but rather uses more resources on producing vast quantities of offspring with less investment per egg. The benefit for this strategy is that the species can produce many offspring, but the cost is that the larvae have a low chance of surviving. The offspring grows fast and do not benefit from any protection by the parents. R-selection is more common among smaller organisms with shorter generation time, and the offspring often becomes sexually mature early compared to K-selected species (Campbell et al., 2015). Whether this reduction of individuals is due to R-selection or just poor development conditions is unknown and further debatable.

Developmental rate

The larval developmental rate was based on the days post hatching to nauplius II and to copepodid stage. The developmental rate across temperatures for the planktonic stages followed the same pattern as the parasitic development, where the developmental rate was slower with colder temperatures and faster with warmer temperatures. Samsing et al. (2016) also presented this in their study of planktonic development of *L. salmonis*, where at 15°C the duration of nauplii stages was 1.8 days while *C. elongatus* development rate to copepodid was 2.5 days. Stien et al. (2005) gathered all developmental studies for salmon lice at the time, and the development from hatching of the eggs to copepodid stage showed the same pattern as our data for *C. elongatus.*

Similar to the hatching success data, a parameter that could influence the data was the actual temperature in incubators. The mean of the manually measured temperature for T6 was 6.2°C, and for T9 it was 9.6°C (Appendix Table 6.2). The two other temperatures were measured to be maintaining the correct temperatures of 12 and 15°C.

4.2.3 Copepodid survival

The copepodid survival results showed that the longest surviving lice were those at colder temperatures. This survival is likely linked to the metabolic rate, and how organisms transform energy and materials. Gillooly et al. (2001), argued how this rate is mainly decided from two closely related processes: the Boltzmann factor, that describes how temperature affects different internal processes, and the quarter-power allometric relation, that describes how different biological processes are scaled by body size. The study shows that the temperature dependence of metabolic rate is relatively constant for unicells, invertebrates and plants. This can be seen in our experiment for lice at colder temperatures, which uses less energy and can therefore survive for a longer period than the lice at warmer temperatures.

The infectivity of salmon lice copepodids is highly linked to its age (Skern-Mauritzen et al., 2020). Infection increases with temperature, while energy stores and activity decrease with time during the copepodid stage, affecting membrane lipid composition (Skern-Mauritzen et al. 2020). Infectivity increased just after molting into copepodids, and thereafter declined with the age of the copepodid for all the experimental temperatures (5, 10 and 15°C; Skern-Mauritzen et al. 2020). Infectivity was not measured in our experiment, but *C. elongatus* most likely has the same pattern as the salmon lice: a longer infection window but with a lower infection pressure for the colder temperature, and a shorter infection window but a higher infection pressure for the warmer temperatures. This is also proven by other models where the predicted infection risk is higher with warmer temperatures due to the increased larval production and the faster generation time (Brooker et al., 2018). The survival of copepodids in our experiment was highly influenced by temperature, where the duration decreased with warmer temperature and increased with colder temperature.

4.3 Morphometric measurements

The results from the morphometric measurements showed that *C. elongatus* were bigger at colder temperatures and smaller at warmer temperatures. This was also observed in a study done by Tully (1989) with *L. salmonis* and *C. elongatus* infestations on farmed Atlantic salmon, where they found that louse size was greater in winter and was negatively correlated with temperature. The temperature was measured in the surface water and since this study was not conducted in laboratory conditions, the temperature would naturally fluctuate. The part of the study conducted for ovigerous female *C. elongatus* was comparable for our measurements

of adult female lice with egg strings (Part 2). The mean length of lice reported by Tully (1989) were slightly larger than our experimental lice, with the largest mean length of ~5.7 mm recorded in January with a water temperature of 7°C. For 6°C the length of their lice was ~5.6 which is the same mean length for our female lice with egg strings. For 15°C their lice were ~4.9 and our lice had a mean length of 4.5 and displayed the same pattern as our study where size is bigger with colder temperatures and smaller with warmer temperatures. However, it is unknown how they measured their lice and the chance they measured their lice with the same procedure as our study is not likely.

The negatively correlated size with temperature was also observed in the study of Samsing et al. (2016) where they reported that all of the morphometric measurements of *L. salmonis* increased with decreasing temperatures. The negatively correlation of size with increasing temperature is found elsewhere in other free-living planktonic copepods (Diaz & Evans, 2008).

Further research

The temperature-dependent development of *C. elongatus* needs to be further studied with robust experimental set ups, as there were some uncertainties in our results. For further research of this lice, there should be around the same number of individuals for each temperature group to ensure good results, so as to not be affected by sample size. The experiment should also be conducted for a broader range of temperatures to find their thermal range. Lastly, it would be interesting to conduct the experiment with the use of another host species, and maybe a more preferred host for *C. elongatus.*

5 Conclusion

There is an increase of reported observations of *C. elongatus* on Atlantic salmon in commercial salmon farms in Norway. It is rather difficult to determine whether this increase comes from an actual increase of their population abundance, or if the industry is becoming more aware of this louse as a different species than the salmon louse and are therefore more likely to report it. Another reason for this increase in the observed amount of *C. elongatus* could be that their population dynamics and host interactions are slightly changing. *C. elongatus* is more prevalent on the wild fish populations than salmon lice, rendering the understanding of population dynamics and general abundance of *C. elongatus* a difficult task.

The larger perspective of increasing temperature in our oceans is studied by Sandvik et al. (2021), where they stated that the infectivity of lice increases with increasing temperature. They concluded that a warmer climate would likely increase the infection pressure of salmon lice from farmed fish to the wild stocks of salmonids. This pattern might be found for *C. elongatus* as well, and further research on their infection pressure is needed.

The overall knowledge generated by this study was that *C. elongatus* development is highly linked to temperature, while the size of adult lice is negatively correlated with temperature. Lower temperatures will lead to a slower development (longer generation time), larger growth of individuals, and higher fecundity. Argued by (1989), this would lead to more intense but separated waves of infection by the sea lice in the winter period. For further expansion of the fish farming industry, we need increased knowledge on many aspects of possible higher lice infestations in coastal waters, particularly as the economic cost of controlling sea lice is substantial for the fish farming industry. The sea lice issue will most likely always be a part of the industry while there are farmed fish in the sea. More knowledge could only lead to better economics of the industry, and less pressure on the wild salmonid stocks.

References

á Nordi, G., Simonsen, K., Danielsen, E., Eliasen, K., Mols-Mortensen, A., Christiansen, D. H., Steingrund, P., Galbraith, M., & Patursson. (2015). Abundance and distribution of planktonic Lepeophtheirus salmonis and Caligus elongatus in a fish farming region in the Faroe Islands. *Aquaculture Environment Interactions*, *7*(1), 15–27. https://doi.org/10.3354/aei00134

Aaen, S. M., Helgesen, K. O., Bakke, M. J., Kaur, K., & Horsberg, T. E. (2015). Drug resistance in sea lice: A threat to salmonid aquaculture. In *Trends in Parasitology* (Vol. 31, Issue 2, pp. 72–81). Elsevier Current Trends. https://doi.org/10.1016/j.pt.2014.12.006

Boxshall, G. A. (1974). Infections With Parasitic Copepods in North Sea Marine Fishes. *Journal of the Marine Biological Association of the United Kingdom*, *54*(2), 355–372. https://doi.org/10.1017/S0025315400058598

Brooker, A. J., Skern-Mauritzen, R., & Bron, J. E. (2018). Production, mortality, and infectivity of planktonic larval sea lice, Lepeophtheirus salmonis (Kroyer, 1837): Current knowledge and implications for epidemiological modelling. *ICES Journal of Marine Science*, *75*(4), 1214–1234. https://doi.org/10.1093/icesjms/fsy015

Campbell, N. A., Reece, J. B., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., & Jackson, R. B. (2015). *Biology A Global Approach* (10th ed.). Pearson Global Edition.

Carrera, E., Garcia, L., Cespedes, A., Gonzalez, I., Sanz, B., Hernandez, P. E., & Martin, R. (1998). Iden1:i'ficationof Atlantic Salmon (Sa/rna sa/ar) and Rainbow Trout (Oncorhynchus rnykiss) by Using Polymerase Chain Reaction Amplification and Restriction Analysis of the Mitochondrial Cytochrome b Gene. In *Journal of Food Protection* (Vol. 61, Issue 4). https://jfoodprotection.org/doi/pdf/10.4315/0362-028X-61.4.482

Costello, M. J. (2006). Ecology of sea lice parasitic on farmed and wild fish. In *Trends in Parasitology* (Vol. 22, Issue 10, pp. 475–483). Elsevier Current Trends. https://doi.org/10.1016/j.pt.2006.08.006

Dalvin, S., Are Hamre, L., Skern-Mauritzen, R., Vågseth, T., Stien, L., Oppedal, F., & Bui, S. (2020). The effect of temperature on ability of Lepeophtheirus salmonis to infect and persist on Atlantic salmon. *Journal of Fish Diseases*, *43*(12), 1519–1529. https://doi.org/10.1111/jfd.13253

Diaz, W., & Evans, F. (2008). Microsetella Norvegica (Boeck): a Direct Relationship Between Seasonal Sea Temperature and Adult Size in a Planktonic Copepod. *Crustaceana*, *34*(3), 313–315. https://doi.org/10.1163/156854078X00880

Forskrift om bekjempelse av lakselus i akvakulturanlegg - Lovdata. (n.d.). Retrieved April 30, 2019, from https://lovdata.no/dokument/SF/forskrift/2012-12-05-1140

Foundation, T. R. (2018). *The R Project for Statistical Computing*. URL: Http://Www. r-Project. Org/. https://www.r-project.org/

Geitung, L., Oppedal, F., Stien, L. H., Dempster, T., Karlsbakk, E., Nola, V., & Wright, D. W. (2019). Snorkel sea-cage technology decreases salmon louse infestation by 75% in a full-cycle commercial test. *International Journal for Parasitology*, *49*(11), 843–846. https://doi.org/10.1016/j.ijpara.2019.06.003

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, *293*(5538), 2248–2251. https://doi.org/10.1126/science.1061967

Hamre, L. A., Bui, S., Oppedal, F., Skern-Mauritzen, R., & Dalvin, S. (2019). Development of the Salmon Louse Lepeophtheirus Salmonis Parasitic Stages in Temperatures Ranging from 3 to 24°C. *Aquaculture Environment Interactions*, *11*, 429–443. https://doi.org/10.3354/aei00320

Hamre, L., Bui, S., Oppedal, F., Skern-Mauritzen, R., & Dalvin, S. (2019). Development of the salmon louse Lepeophtheirus salmonis parasitic stages in temperatures ranging from 3 to 24°C. *Aquaculture Environment Interactions*, *11*, 429–443. https://doi.org/10.3354/aei00320

Hemmingsen, W., MacKenzie, K., Sagerup, K., Remen, M., Bloch-Hansen, K., & Dagbjartarson Imsland, A. K. (2020). Caligus elongatus and other sea lice of the genus Caligus as parasites of farmed salmonids: A review. In *Aquaculture* (Vol. 522, p. 735160). Elsevier B.V. https://doi.org/10.1016/j.aquaculture.2020.735160

Heuch, P. A., Øines, Knutsen, J. A., & Schram, T. A. (2007). Infection of wild fishes by the parasitic copepod Caligus elongatus on the south east coast of Norway. *Diseases of Aquatic Organisms*, *77*(2), 149–158. https://doi.org/10.3354/dao01833

Heuch, P. A., & Schram, T. A. (1999). Crustacea (krepsdyr). In T. Poppe (Ed.), *Fiskehelse og fiskesykdommer*.

Hogans, W. E., & Trudeau, D. J. (1989). Preliminary studies on the biology of sea lice, caligus elongatus, caligus curtus and lepeophtheirus salmonis. *Canadian Technical Report of Fisheries and Aquatic Sciences*, 1715. https://www.cabi.org/isc/abstract/19900597424

Johnson, S. C., & Albright, L. J. (1991). Development, growth, and survival of lepeophtheirus salmonis (Copepoda: Caligidae) under laboratory conditions. *Journal of the Marine Biological Association of the United Kingdom*, *71*(2), 425–436. https://doi.org/10.1017/S0025315400051687

Kabata, Z. (1979). *Parasitic Copepoda of British Fishes*.

Kabata, Z. (1992). *Copepods parasitic on British fishes*.

Kabata, Z., & Hewitt, G. C. (1971). Locomotory Mechanisms in Caligidae (Crustacea: Copepoda). *Journal of the Fisheries Research Board of Canada*, *28*(8), 1143–1151. https://doi.org/10.1139/f71-169

Kaji, T., Venmathi Maran, B. A., Kondoh, Y., Ohtsuka, S., Boxshall, G. A., & Tsukagoshi, A. (2012). The lunule of caligid copepods: An evolutionarily novel structure. *Evolution and Development*, *14*(6), 465–475. https://doi.org/10.1111/ede.12000

Karlsbakk, E., Nylund, A., & Nilsen, F. (2019). *Fiskeparasitter* (pp. 7–17).

Mustafa, A., Mackinnon, B. M., & Piasecki, W. (2005). Interspecific differences between Atlantic salmon and Arctic charr in susceptibility to infection with larval and adult Caligus elongatus: Effect of skin mucus protein profiles and epidermal histological differences. *Acta Ichthyologica et Piscatoria*, *35*(1), 7–13. https://doi.org/10.3750/AIP2005.35.1.02

Nordmann, A. Von. (1832). *Mikrographische Beiträge zur Naturgeschichte der Wirbellosen Thiere. Zweites heft (1+2 part)* (G. Reimer (Ed.)).

Øines, & Heuch, P. A. (2007). Caligus elongatus Nordmann genotypes on wild and farmed fish. *Journal of Fish Diseases*, *30*(2), 81–91. https://doi.org/10.1111/j.1365-2761.2007.00783.x

Øines, Ø., & Heuch, P. A. (2005). Identification of sea louse species of the genus Caligus using mtDNA. *Journal of the Marine Biological Association of the United Kingdom*, *85*(1), 73–79. https://doi.org/10.1017/S0025315405010854h

Øines, Simonsen, J. H., Knutsen, J. A., & Heuch, P. A. (2006). Host preference of adult Caligus elongatus Nordmann in the laboratory and its implications for Atlantic cod aquaculture. *Journal of Fish Diseases*, *29*(3), 167–174. https://doi.org/10.1111/j.1365-2761.2006.00702.x

Overton, K., Barrett, L. T., Oppedal, F., Kristiansen, T. S., & Dempster, T. (2020). Sea lice removal by cleaner fish in salmon aquaculture: A review of the evidence base. *Aquaculture Environment Interactions*, *12*, 31–44. https://doi.org/10.3354/aei00345

Parker, R. R. (1969). Validity of the Binomen Caligus elongatus for a Common Parasitic Copepod Formerly Misidentified with Caligus rapax. *Journal of the Fisheries Research Board of Canada*, *26*(4), 1013–1035. https://doi.org/10.1139/f69-097

Piasecki, W. (1996). The developmental stages of Caligus elongatus von Nordmann, 1832 (Copepoda: Caligidae). *Canadian Journal of Zoology*, *74*(8). https://doi.org/10.1139/z96-161

Piasecki, W., & Mackinnon, B. M. (1995). Life cycle of a sea louse, Caligus elongatus von Nordmann, 1832 (Copepoda, Siphonostomatoida, Caligidae). *Canadian Journal of Zoology*, *73*(1), 74–82. https://doi.org/10.1139/z95-009

Piasecki, W., & MacKinnon, B. M. (1993). Changes in structure of the frontal filament in sequential developmental stages of Caligus elongatus von Nordmann, 1832 (Crustacea, Copepoda, Siphonostomatoida) . *Canadian Journal of Zoology*, *71*(5), 889–895. https://doi.org/10.1139/z93-116

Pike, A. W., Mordue, A. J., & Ritchie, G. (1993). The development of Caligus elongatus Nordmann from hatching to copepodid in relation to temperature. In *Pathogens of Wild and Farmed Fish: Sea Lice* (pp. 53–60). Ellis Horwood Limited.

Pike, A. W., & Wadsworth, S. L. (1999). Sealice on salmonids: Their biology and control. In *Advances in Parasitology* (Vol. 44, pp. 233–337). Academic Press. https://doi.org/10.1016/s0065-308x(08)60233-x

Powell, A., Treasurer, J. W., Pooley, C. L., Keay, A. J., Lloyd, R., Imsland, A. K., & Garcia de Leaniz, C. (2018). Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. In *Reviews in Aquaculture* (Vol. 10, Issue 3, pp. 683–702). John Wiley & Sons, Ltd. https://doi.org/10.1111/raq.12194

Samsing, F., Oppedal, F., Dalvin, S., Johnsen, I., Vågseth, T., & Dempster, T. (2016). Salmon lice (Lepeophtheirus salmonis) development times, body size, and reproductive outputs follow universal models of temperature dependence. *Canadian Journal of Fisheries and Aquatic Sciences*, *73*(12), 1841–1851. https://doi.org/10.1139/cjfas-2016-0050

Sandvik, A. D., Dalvin, S., Skern-Mauritzen, R., & Skogen, M. D. (2021). The effect of a warmer climate on the salmon lice infection pressure from Norwegian aquaculture. *ICES Journal of Marine Science*. https://doi.org/10.1093/icesjms/fsab069

Skern-Mauritzen, R., Sissener, N. H., Sandvik, A. D., Meier, S., Sævik, P. N., Skogen, M. D., Vågseth, T., Dalvin, S., Skern-Mauritzen, M., & Bui, S. (2020). Parasite development affect dispersal dynamics; infectivity, activity and energetic status in cohorts of salmon louse copepodids. *Journal of Experimental Marine Biology and Ecology*, *530–531*, 151429. https://doi.org/10.1016/j.jembe.2020.151429

Stien, A., Bjørn, P. A., Heuch, P. A., & Elston, D. A. (2005). Population dynamics of salmon lice Lepeophtheirus salmonis on Atlantic salmon and sea trout. In *Marine Ecology Progress Series* (Vol. 290, pp. 263–275). Inter-Research. https://doi.org/10.3354/meps290263

Stien, L. H., Nilsson, J., Hevrøy, E. M., Oppedal, F., Kristiansen, T. S., Lien, A. M., & Folkedal, O.

(2012). Skirt around a salmon sea cage to reduce infestation of salmon lice resulted in low oxygen levels. *Aquacultural Engineering*, *51*, 21–25. https://doi.org/10.1016/J.AQUAENG.2012.06.002

Trafikklyssystemet – HI sin kunnskap | Havforskningsinstituttet. (n.d.). Retrieved September 4, 2021, from https://www.hi.no/hi/temasider/akvakultur/trafikklyssystemet-hi-sin-kunnskap

Treasurer, J. W., & Bravo, S. (2011). The spatial distribution patterns of Caligus rogercresseyi and C. elongatus on Atlantic salmon hosts (Salmo salar). *Aquaculture*, *320*(3–4), 154–158. https://doi.org/10.1016/j.aquaculture.2011.03.032

Tully, O. (1989). The Succession of Generations and Growth of the Caligid Copepods Caligus Elongatus and Lepeophtheirus Salmonis Parasitising Farmed Atlantic Salmon Smolts (Salmo Salar L.). *Journal of the Marine Biological Association of the United Kingdom*, *69*(2), 279–287. https://doi.org/10.1017/S0025315400029404

Walter, T. C., & Boxshall, G. (2021). *World of Copepods*. https://doi.org/doi:10.14284/356

Wootten, R., Smith, J. W., & Needham, E. A. (1982). Aspects of the biology of the parasitic copepods Lepeophtheirus salmonis and Caligus elongatus on farmed salmonids, and their treatment. *Proceedings of the Royal Society of Edinburgh. Section B. Biological Sciences*, *81*(3), 185–197. https://doi.org/10.1017/s0269727000003389

6 Appendix

Table 6.1: Sampling overview and lice counts. DPI: Days past infection with Caligus elongatus, Ch3: chalimus 3, Ch4: chalimus 4, AttM: attached male, AttF: attached female, AM: adult male, AF: adult female, AF1: first egg strings, AF2: second egg strings. 10 fish were examined each sampling.

Infection (day.month.year,time)	Sampling (day.month.year,time)	Temp. (°C)	DPI	Tank	Sampling Nr.	Ch2	Ch3	Ch4	AttM	AttF	AM	AF	AF1	AF2	SumTot
01.08.2020, 20:00	04.09.2020 09:05	6	33,5	1	1		2	14			1				17
01.08.2020, 20:00	09.09.2020 09:10	6	38,5	2	2			7			8				15
01.08.2020, 20:00	14.09.2020 12:10	6	43,7	3	3						4	3			7
01.08.2020, 20:00	22.09.2020 08:15	6	51,5	1	4						7	8			15
01.08.2020, 20:00	29.09.2020 20:00	6	59,0	2	5						1	1	1	2	5
01.08.2020, 20:00	07.10.2020 15:00	6	66,8	3	6						2			2	4
01.08.2020, 20:00	09.10.2020 14:10	6	68,8	1,2	7						3	1	2	8	14
01.08.2020, 20:00	17.10.2020 11:20	6	76,6	1,2,3	8						1	1	1	9	12
01.08.2020, 20:00	21.08.2020 13:00	9	19,7	6	1	1	19	80							100
01.08.2020, 20:00	24.08.2020 14:30	9	22,8	7	2			16	3	9	12	1			41
01.08.2020, 20:00	28.08.2020 09:20	9	26,6	8	3			1	4	5	36	21			67
01.08.2020, 20:00	01.09.2020 12:20	9	30,7	6	4						36	24	5		65
01.08.2020, 20:00	06.09.2020 10:10	9	35,6	7	5						8	10	1		19
01.08.2020, 20:00	10.09.2020 16:00	9	39,8	8	6						20	1	1	33	55
01.08.2020, 20:00	13.09.2020 18:05	9	42,9	6	7						25	2	2	21	50
01.08.2020, 20:00	17.09.2020 08:20	9	46,5	7	8						3	4	1	12	20
08.08.2020, 19:00	22.08.2020 11:00	12	13,7	9	1		3	4							7
08.08.2020, 19:00	24.08.2020 14:00	12	15,8	10	2			9		1	1	1			12
08.08.2020, 19:00	26.08.2020 11:00	12	17,7	11	3				2	2	4	4			12
08.08.2020, 19:00	29.08.2020 14:10	12	20,8	9	4					1	6	4	2		13
08.08.2020, 19:00	01.09.2020 13:10	12	23,8	10	5						6	1		8	15
08.08.2020, 19:00	04.09.2020 14:20	12	26,8	11	6						2	1	1	4	8
08.08.2020, 19:00	06.09.2020 17:00	12	28,9	9	7						4	2		16	22
08.08.2020, 19:00	08.09.2020 17:00	12	30,9	10	8						2		1	5	8
08.10.2020, 14:00	18.10.2020 11:00	15	9,9	13	1		1								1
08.10.2020, 14:00	19.10.2020 20:00	15	11,3	14	2			2	1		2	2			7
08.10.2020, 14:00	21.10.2020 08:15	15	12,8	15	3			2			8	10			20
08.10.2020, 14:00	23.10.2020 13:30	15	15,0	14	4						1	5			6
08.10.2020, 14:00	25.10.2020 20:00	15	17,3	15	5						5	2	9		16
08.10.2020, 14:00	28.10.2020 10:30	15	19,9	14,15	6						6		5	5	16
08.10.2020, 14:00	30.10.2020 08:40	15	21,8	15	7						1	1	1	7	10

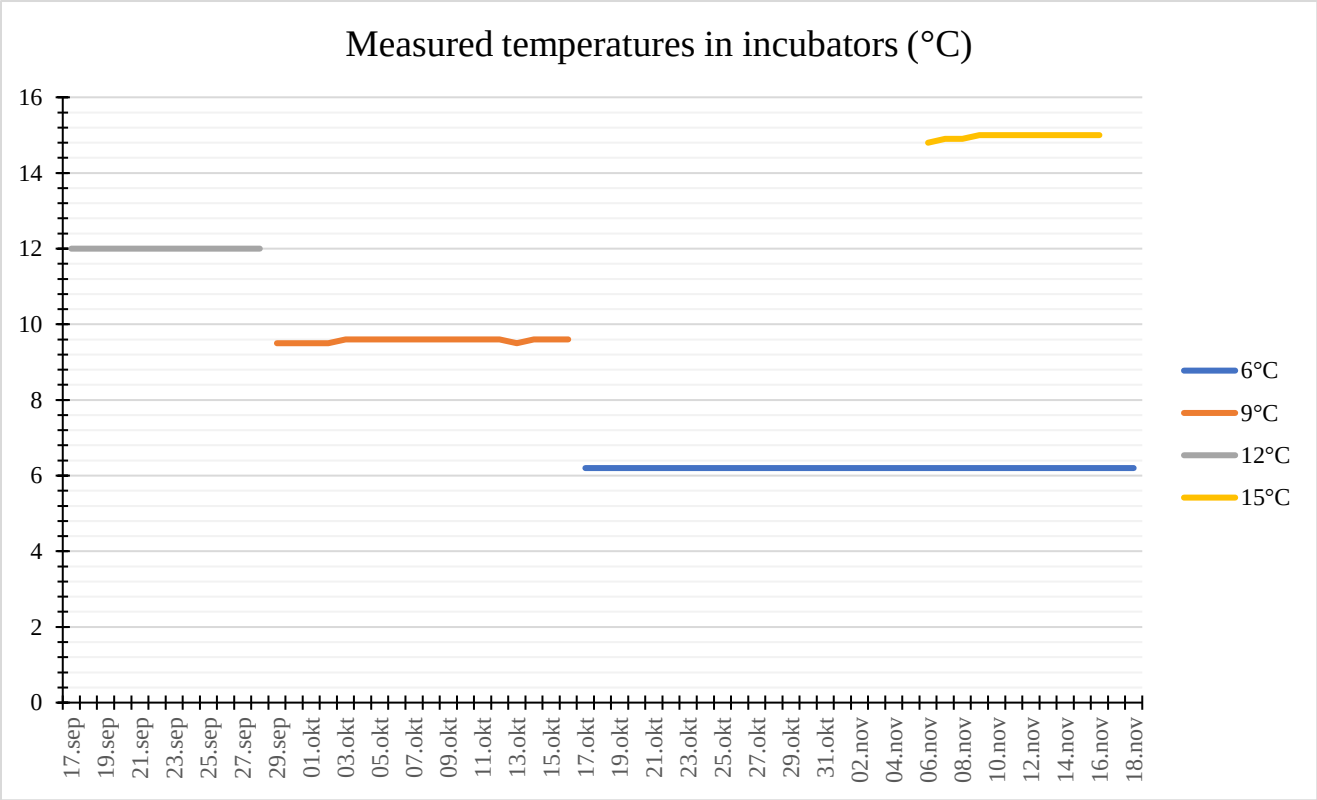

Fig: 6.1: Mean measured temperature in the incubators and larval development of part 2 of the experiment. Manually measured with a thermometer for the water going into the individual boxes.