

MASTER THESIS

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Oxygen consumption of the sea lice *Lepeophtheirus salmonis* and *Caligus elongatus* life stages at different temperatures

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Abstract

Infestation by sea lice is the leading disease challenge limiting the production of Atlantic salmon and can result in reduced production and profitability as well as reduced fish welfare. Identifying factors that influence the population dynamics of sea lice is critical for predicting and managing the impacts of epidemics, as well as managing sea lice in aquaculture farms. Increases in mean sea temperature, as is happening due to climate change, may increase the abundance of sea lice because of shorter generation times and increased reproduction success. However, if the optimal temperature for growth and survival is exceeded, the occurrence of sea lice may decrease.

To investigate the effects of ocean warming on the temperature sensitivity and physiological performance of sea lice, the impact of short-term temperature acclimation on standard metabolic rate was measured. A fluorescence-based microplate respirometer was used to measure the oxygen consumption of *Lepeophtheirus salmonis* and *Caligus elongatus* in five different thermal regimes (4, 8, 12, 16, and 20°C).

As expected, temperature significantly affected the oxygen consumption rate in *L. salmonis*. Oxygen consumption increased with body length, life stage, and temperature in the study. The absence of significant differences in oxygen consumption in *C. elongatus* can be attributed to a low sample size at few temperatures. In *L. salmonis*, the lowest oxygen consumption of the test temperatures was at 12°C, which might indicate an optimal temperature for *L. salmonis*. Animals tested at 20°C had the highest oxygen consumption, suggesting that they may have been stressed and at or beyond their upper thermal tolerance. The results from this study can be used to predict the responses of sea lice to altered sea temperatures following climate change.

1.0 Introduction

1.1 Temperature and metabolic rate in marine ectotherms

All organisms consume energy to survive and grow, and the energy consumption can be estimated by measuring the animals' metabolic rate under given conditions (Gillooly et al., 2001). The metabolic rate can be determined by the amount of oxygen consumed by an animal's cellular respiration and measured indirectly through measures of oxygen consumption (Evans and Heather, 2016). Oxygen is important in several oxidative activities releasing energy and is therefore crucial for metabolism. The oxygen requirements and oxygen consumption rates of a species depend on several factors, including age, sex, size, activity, temperature, and nutrition (Gillooly et al., 2001, Evans and Heather, 2016, Brett and Groves, 1979). The rate at which organisms transform energy and materials enables growth and reproduction and as a consequence of this, determines the dispersal of organisms (Gillooly et al., 2001, Samsing et al., 2016). Most aquatic animals are ectotherms, where their metabolic rate and energy expenditure are strongly affected by the surrounding water temperature (Karvonen et al., 2010), where higher temperatures in general leads to higher metabolic rate and vice versa. In general, for ectotherms high metabolic rates give a faster growth, earlier reproduction, and shorter life span. In contrast, low metabolic rates are associated with slow growth, late reproduction, and a long lifespan (Angilletta Jr et al., 2004, Magnuson et al., 1997).

Organisms typically have an upper and lower tolerance limit, where the species within these limits work at their optimal or close to optimal, and the requirements for growth and reproduction are met (Hofmann and Todgham, 2010, Fry, 1971). Thus, temperature directly affects the rate of all biological processes, including the oxygen consumption of aquatic species. This boundary between the minimum and maximum temperature varies with acclimatization, availability of oxygen, and synergistic effects from contamination in water (Newell and Branch, 1980). The optimal temperature is usually near but below the warm edge of its limits, where the aerobic scope, cardiovascular function, growth, and lifetime fitness are best utilized (Jobling, 1996, Martin and Huey, 2008). The thermal tolerance and optimal temperature might differ within a species, depending on life stage and developmental process. Temperatures outside these limits can lead to lower capacity to maintain optimal enzymatic activities, affecting feeding, growth, and reproduction, and can in turn be detrimental to the animal (Jobling, 1996).

Temperature is therefore also a strong determinant of geographic ranges (Calosi et al., 2010), and altered temperatures can cause changes in the biogeographic distribution of species

(Beaugrand et al., 2002, Parmesan and Yohe, 2003). Due to increased anthropogenic activity, higher CO₂ levels in the atmosphere have caused global sea surface temperature to increase by an average of 1.1°C from preindustrial times. This temperature rise is expected to continue rising by 1-4°C by the end of the century, and the Norwegian coast is warming above the global average (IPCC, 2014, IPCC, 2022).

1.2 Sea lice

Sea lice is a general term for ectoparasitic copepods of the family *Caligidae*. There are over 500 species of caligid sea lice, where *Lepeophtheirus salmonis* (Krøyer, 1837) and *Caligus elongatus* (Nordmann, 1832) are the major species impacting salmonid farming in the North Atlantic (Pike, 1989, Costello, 2006). Depending on the level of host infestation, sea lice can lead to skin irritation and ulcerations (Fig. 1). This can in turn lead to secondary infections, osmoregulatory problems, and in some cases mortality, increasing production costs and reducing the market value of farmed fish (Liu and vanhauwaer Bjelland, 2014, Pike and Wadsworth, 1999).



Figure 1. Infestation of Atlantic salmon by *Lepeophtheirus salmonis*. High infestations will strip the mucosa off the skin, and the parasites start to feed directly on the skin, plasma, and subcutaneous tissue (Photo: Tuva Sørgaard).

Infestation by sea lice is the leading cause of disease that challenges and limits the production of Atlantic salmon (*Salmo salar*) and has been an issue in salmonid farming since the start of the industry in the late 1960s. The concentration of hosts in intensive fish farming increases infestation pressure on both farmed and wild fish (Serra-Llinares et al., 2014, Jansen et al.,

2012) and has been associated with declines in wild salmonid populations (Krkošek et al., 2013, Gargan et al., 2003). Since the 1960s, the Norwegian fish farming industry has enjoyed remarkable expansion thanks to growth opportunities such as location dispensations allocated by the authorities, together with innovations that have improved production and resulted in lower costs. However, a further increase in production is limited by governmental regulations regarding *L. salmonis* potential impact on migrating smolts (Taranger et al., 2015). For the fish farmers, the costs of sea lice treatment cause significant economic effects and influence public perceptions of aquaculture (Costello, 2009, Torrissen et al., 2013). The costs of sea lice infestations include lower slaughter weights, increased feed costs, investments in lice control, treatment costs, and additional labor costs. The direct costs for control, prevention, and treatment of sea lice infestations in Norwegian salmonid aquaculture were estimated by Iversen et al. (2017) to be close to NOK 5 billion per year.

1.2.1 Lepeophtheirus salmonis

Lepeophtheirus salmonis is the dominating species on salmonid farms, causing the greatest damage to cage-culture populations globally (Pike and Wadsworth, 1999) and in Norway (Asplin et al., 2011). The louse is a parasite of the genera *Salmo*, *Salvelinus*, and *Oncorhynchus* in the Northern hemisphere (Kabata, 1979). Potential wild sources of *L. salmonis* in Norwegian water are Atlantic salmon, sea trout (*Salmo trutta*), and Arctic charr (*Salvelinus alpinus*). The louse has a direct life cycle with eight life stages separated by a molt; two nauplii stages, one copepodid stage, two chalimii stages, and two preadult stages, followed by the fully mature adult stage (Fig. 2).

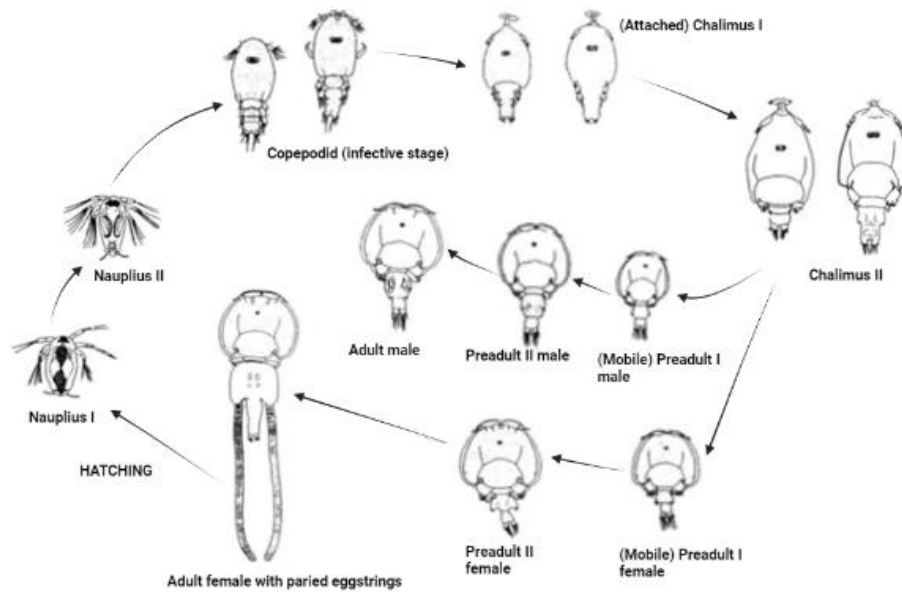


Figure 2. The life cycle of *Lepeophtheirus salmonis*. The species was previously mistaken for having ten life stages (Johnson and Albright, 1991b, Schram, 1993), but this was proven wrong by Hamre et al. (2013). Modified from Schram (1993).

The three first life stages of *L. salmonis* are planktonic, dependent on internal nutrient reserves for energy to perform basic metabolism, development, and locomotion (Tucker et al., 2000b). The nauplii larvae are dispersed into the water during hatching, drifting passively using the current to spread the parasite (Asplin et al., 2011). The nauplii are almost translucent with distinct pigment regions of black and brown pigment (Johnson and Albright, 1991b). The newly hatched larvae have a short cylindrical shape of 0.5 mm in length, which elongates shortly after (Schram, 1993, Johannessen, 1977). The nauplii use brushed legs to swim and stay in the upper water layers allowing it to adjust its positions vertically (Johannessen, 1977). The abundance and distribution of planktonic sea lice are highly variable due to varying environmental conditions in fjord systems, and the planktonic larvae can potentially be transported 200 km over 10 days (Asplin et al., 2011), contributing to an elevated infestation pressure over a large geographic area.

As the nauplii develop from the early to mid-phase of nauplii stage I, it shows phototaxis behavior moving toward light and developing more pigment. The second nauplii stage has a similar appearance to the previous stage but is more elongated, with a mean length of 0.6 mm (Schram, 1993, Johnson and Albright, 1991b). The concentration of yolk has decreased, while the pigmented areas have increased. The nauplii stage II lasts longer than nauplii stage I, due

to the extensive metamorphosis from nauplii to the copepodid stage (Johnson and Albright, 1991b, Pike and Wadsworth, 1999).

The copepodid is elongated with a hydrodynamic shape, with a length of ca. 0.7 mm (Schram, 1993). The body of the copepodid is segmented, allowing it to be a more active swimmer than in the previous stages (Johannessen, 1977). In this stage, the lice still have some yolk left but are dependent on infesting a suitable host to continue the life cycle. Finding the right host is time-limited by endogenous energy reserves (Tucker et al., 2000b). The infestation window for *L. salmonis* is estimated to be from 90 to 140 degree days, with a peak infestivity 1-2 days after molting to the infestive stage before declining towards the end of their life expectancy (Skern-Mauritzen et al., 2020, Samsing et al., 2016). The lice try to enhance their chances of meeting and recognizing hosts by adjusting their position in the water column in response to external stimuli such as positive phototaxis (Heuch et al., 1995, Johannessen, 1977, Bron et al., 1993, Costello, 2006), flickering light (Fields et al., 2018), mechanical vibration generated by the host (Bron et al., 1993, Heuch and Karlsen, 1997), or chemical cues (Bailey et al., 2006). Temperature and salinity play an essential role in the successful settlement, survival, and development of *L. salmonis* copepodid on the host (Tucker et al., 2000a, Samsing et al., 2016). When the copepodid has encountered a host, it initially settles on the fish using the second antennae and maxillipeds before molting to the attached chalimus (Schram, 1993). The body length of the chalimus stage range from 1.1 mm to 2.3 mm. The louse extrudes a frontal filament to securely attach to the host, penetrating the epidermal tissues and anchoring it to the basement membrane. The filament attachment continues until preadult I and restricts the louse's feeding area by the length of the filament.

The lice move around the skin surface at the mobile stages called preadult I, II, and adult, causing more extensive damage. Clinical signs of infestation are related to the development stage of the lice, the number of lice on fish, host size, age, and general state (Pike and Wadsworth, 1999). *L. salmonis* is thought to damage more than *C. elongatus* per parasite individual because of its size, all-year presence, and preference to attach to areas with a thinner epidermis (Pike and Wadsworth, 1999, Costello, 2006). Adult males and preadult II females of *L. salmonis* can change hosts when the distance is short, which is thought to increase the possibility of successful mate location (Hull et al., 1998, Ritchie, 1997). The length of the preadult is 3.4-5.2 mm long, depending on sex (Schram, 1993). The adult female is 8-12 mm long, and the male is 5-6 mm (Schram, 1993). Differences in the size of adult *L. salmonis* have been reported, thought to be due to sampling location, season, and if the lice are wild or farmed

(Pike and Wadsworth, 1999). The adult differs from the preadult stages in the shape of genital and abdominal segments (Johnson and Albright, 1991b). In females the genital segment has a square shape, while in males it is barrel-shaped (Fig. 3). Copulation occurs between an adult male and a recently molted female. The females gradually release spermatophores from the adult male to fertilize eggs coming from the ovaries, allowing them to produce multiple egg strings from a single copulation (Ritchie et al., 1996). Egg sac length and the number of eggs per sac are related and vary widely between individuals. The eggs per sac can range from 100 to 700 eggs, and *L. salmonis* can produce at least six sets of egg strings (reviewed in Pike and Wadsworth, 1999, Ritchie et al., 1993).

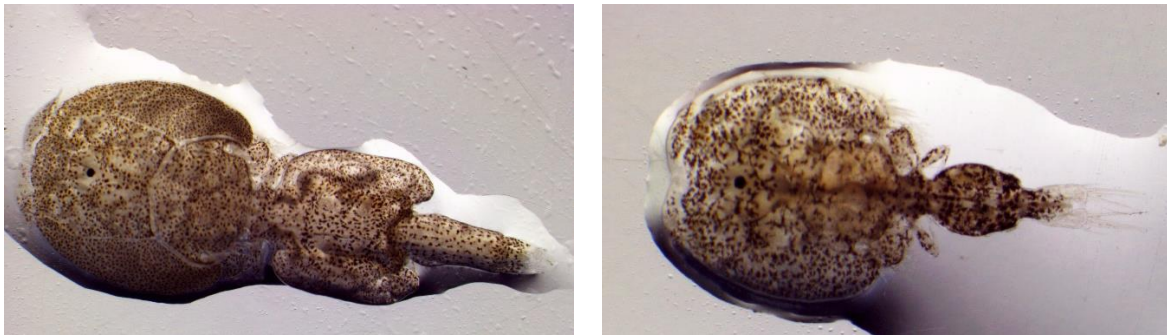


Figure 3. Adult female (left) and male (right) of *Lepeophtheirus salmonis* (Photo: Tuva Sørgaard).

1.2.2 *Caligus elongatus*

Caligus elongatus is smaller than *L. salmonis*, has a light yellow-brown color along the edges, and is darker in the middle (Fig. 4) (Hogans and Trudeau, 1989). The parasitic copepod has been reported under different names and incorrectly reported from most regions of the world's oceans (Parker, 1969, Kabata, 1979). Today the species are considered most abundant in the North Atlantic (Hemmingsen et al., 2020). The lice have a broad host range (Parker, 1969, Boxshall, 1974, Kabata, 1979) and the ability to become temporarily planktonic, allowing for a switch of a host (Bruno and Stone, 1990). The life cycle of *C. elongatus* consists of eight stages separated by molts: nauplius (I and II), copepodid, chalimus (I-IV), and adult (Piasecki, 1996, Piasecki and MacKinnon, 1995). The life cycle of *C. elongatus* is similar to *L. salmonis*, but unlike *L. salmonis*, *C. elongatus* does not have preadult stages (Piasecki and MacKinnon, 1995, Piasecki, 1996).



Figure 4. Adult *Caligus elongatus* with paired egg strings (Photo: Tuva Sørgaard).

The lice are a known problem for the Atlantic salmon farming industry (Pike and Wadsworth, 1999, McKenzie et al., 2004). They have been recorded from farmed fish in Norway (Bristow and Berland, 1991), Scotland (Wootten et al., 1982), Canada (Hogans and Trudeau, 1989), Ireland (Jackson and Minchin, 1992), and the Faroes (á Norði et al., 2015). *Caligus elongatus* is found on more than 80 different host species, both teleost and elasmobranch species (Parker, 1969, Boxshall, 1974, Kabata, 1979).

Wild fish are considered an essential source of infestation with *C. elongatus* in farmed salmonids (Jackson et al., 2000, Bruno and Stone, 1990). Many of the natural hosts of *C. elongatus* congregate around fish farms to feed on wasted feed, and sudden rises in adult *C. elongatus* in Atlantic salmon farming can come from passing wild hosts, such as the saithe and herring (MacKenzie and Morrison, 1989, Bruno and Stone, 1990, Wootten et al., 1982). Free-living adults of *C. elongatus* have been found in the plankton (Neilson et al., 1987), and Øines et al. (2006) demonstrated that adult *C. elongatus* could reinfest other fish species after being taken off the original host. *Caligus elongatus* has a greater variety and quantity of protease enzymes than *L. salmonis*, which allows it to handle a broad range of chemical substrates (Ellis et al., 1990), possibly enabling its broad host specificity. In Norwegian waters, there are two genotypes of *C. elongatus* referred to as genotype 1 and genotype 2 (Øines and Heuch, 2005). The two genotypes might represent different, near-related species (Øines and Heuch, 2005), where the distribution of the genotypes varies with the season (Heuch et al., 2007) and fish species (Øines et al., 2006).

The focus of research into the impacts of sea lice on salmon aquaculture has remained primarily on *L. salmonis*, even though *C. elongatus* can be problematic for cultured fish (á Norði et al., 2015). Data from Todd et al. (2006) show that *C. elongatus* infestation of wild salmon can in some years equal that of *L. salmonis*.

1.2.3 Sea lice and temperature

The upper 10-20 m depths in Norwegian waters range between 1 and 5°C in winter and can exceed 20°C in summer (Asplin et al., 2011). Here the sea lice experience a wide range of temperatures, including fast changes for the planktonic stages as they move through thermoclines and for the parasitic stages as the host fish move in the water (Dalvin et al., 2020). The sea temperature is a strong influencer of biological processes in sea lice and a critical factor in sea lice epidemiology (Samsing et al., 2016). Schram et al. (1998) found that the prevalence (Percent of a sample group that is infested) and median intensity (Number of lice per unit of fish body weight) of both species increased in the spring and declined in the winter. The development of both the planktonic and the attached stages of *L. salmonis* and *C. elongatus* is temperature-sensitive, speeding up the development rate at high temperatures and slowing down at low temperatures (Samsing et al., 2016, Johannessen, 1977, Tully, 1989, Johnson and Albright, 1991a, Hamre et al., 2019, Pike, 1993). The duration of the egg-bearing period (Johnson and Albright, 1991a, Boxaspen and Næss, 2000, Pike, 1993), hatching success (Samsing et al., 2016, Stien et al., 2005), infestation success (Tucker et al., 2000a, Skern-Mauritzen et al., 2020, Samsing et al., 2016), post-infestation survival (Dalvin et al., 2020), body size and fecundity (Tully, 1989), are all temperature-dependent. Groner et al. (2016) found a generation time for *L. salmonis* as low as 15 days at 16°C, while at 4°C it was as high as 100 days, suggesting that infestations may increase in response to warmer temperatures. Together with the increased survival, infestation success, and decreased generation time in warmer temperatures, it is suggested that the infestation pressure of sea lice is more substantial in summer than in winter (Ritchie et al., 1993).

The optimal thermal range for salmon lice is not fully elucidated (Overton et al., 2019). A study by Hamre et al. (2019) found that the development of *L. salmonis* from infestation to the adult stage was severely compromised at 3 and 24°C, while the lice developed as normal in the temperature range from 6 to 21°C. Sandvik et al. (2021) noted that increased larvae production, decreased developmental time from non-infective nauplii to infectious copepodids, and higher infestations of copepodids will lead to adverse effects in a warmer climate and are likely to increase the infestation pressure of lice.

1.3 Research aim

As with all copepods, sea lice have preferred environmental conditions determined by their physiological tolerances (Brooker et al., 2018). For marine ectotherms sea temperature regulates the metabolism, which can be determined by the amount of oxygen consumed by its cellular respiration and measured indirectly through measures of oxygen consumption (Evans and Heather, 2016, Fry, 1971, Brett and Groves, 1979). Studies on *L. salmonis* (Thompson et al., 2019) and *Caligus rogercresseyi* (Montory et al., 2020), as well as on other marine copepods show that the metabolic rate increase with temperature (Isla and Perissinotto, 2004, Almeda et al., 2011, Childress, 1977). The thermal window of a species can be used in species distribution modeling and predict response to climate change (Pearson and Dawson, 2003, Sunday et al., 2012).

There has been extensive study on *L. salmonis* to better understand how infestations can be reduced in fish populations. Still, there is far less information on the epidemiology of *C. elongatus* (Revie et al., 2002a, á Norði et al., 2015), which is also a challenge for the industry, especially in northern Norway. For parasites with severe consequences to the fish farming industry (Torrissen et al., 2013), more detailed knowledge of their temperature-dependent metabolism is required. Sea temperatures significantly affect the outcome of sea lice infestations and should be considered in the management and risk assessment of the parasites (Dalvin et al., 2020). The study of temperature effect on oxygen consumption will enable farmers to better predict when parasitic intensity is likely to rise. The aim of this study was to examine the thermal tolerance of *L. salmonis* and *C. elongatus*, two common species of sea lice found along the Norwegian coast where salmon farms are present. The temperature dependence of metabolic rate in sea lice was estimated from respiration rate measurements. The hypothesis was that there would be a difference in oxygen consumption between life stages and species of sea lice exposed to different temperatures.

2.0 Material and methods

The experiments were conducted at Matre Research Station, Institute of Marine Research, Norway, over a time period of two months in the autumn of 2021. All experimental procedures were conducted in accordance with the Norwegian regulation on animal experimentation (Forskrift om forsøk med dyr) and were approved by the Norwegian animal research authority (Forsøksdyrutvalget; FOTS ID #27999).

2.1 Experimental animals

2.1.1 Lepeophtheirus salmonis

Larval stages of *L. salmonis* were produced by collecting egg strings from a stock population at Matre research station. Adult *L. salmonis*, which originated from various farms in Masfjorden, were kept on Atlantic salmon (AquaGen strain) held in flow-through seawater tanks. To collect egg strings fish were anesthetized with metomidate hydrochloride (0.01 g/l metomidate hydrochloride, Aquacalm®), which has been observed to have a more positive effect on lice survival than other anesthesia (S. Bui, personal communication). After anesthesia, lice were carefully removed using tweezers and the egg strings were separated from female lice. The egg strings were placed in small incubators suitable for single pairs of egg strings, as described by Hamre et al. (2009) for acclimation (Fig. 5). The incubators had mesh at the bottom, allowing for constant water exchange while preventing animals from escaping (Fig. 5 and Fig. 6).

The egg strings were checked for hatching twice a day (morning and afternoon). The lice were incubated under continuous light to minimize the disturbance and keep the conditions equal for all lice during the acclimation time prior to respirometry tests. The mean temperature during incubation was 12°C (± 1). The lice development was tracked as described by Hamre et al. (2019), using a combination of salmon lice description by Schram (1993) and morphometric data.



Figure 5. Incubators for egg strings and the planktonic stages of sea lice. The incubators allow for water exchange by allowing filtered seawater to enter slowly through the lid, flow through the 16 chambers, and exit through the mesh bottom of the incubator (Photo: Tuva Sørgaard).



Figure 6. Incubators for the larger life stages of sea lice. The incubators have mesh at the bottom, which allows for water exchange. The two incubators are placed in each level tank, which is a regular container without mesh. Filtered seawater runs through the tubes and into the incubators (Photo: Tuva Sørgaard).



Figure 7. Collection of adult *L. salmonis* with egg strings from the stock population at Matre Research Station. The host fish was terminated in connection with another experiment at the research station (Photo: Tuva Sørsgaard).



Figure 8. Experimentally infested fish with preadults of *L. salmonis* (Photo: Tuva Sørsgaard).

Sixty Atlantic salmon post-smolts (AquaGen strain, average weight = 366 g) were stocked in four holding tanks ($1 \times 1 \times 0.6$ m; volume ≈ 0.6 m³) for experimental infestation of *L. salmonis*. The copepodids that hatched from the collected egg strings were then used to infest these fish and produce chalimus, preadult, and adult stages with new egg strings (Fig. 7 and Fig. 8). The copepodid density used for infestations was estimated by collecting the incubator volume, transferring 20 ml into a counting chamber, and enumerating the quantity of viable copepodids in the aliquot. In order to generate the desired infestation pressure of lice, this was repeated five times to acquire an average number of copepodids in the total volume. In total ~600 copepodids were introduced to each tank, which was estimated to give a load of ~30 salmon lice per fish. Fish were infested by stopping the inlet water and lowering the water level to half of the original level, where the copepodids were distributed evenly. After 40 min, the water flow was re-

instated. Two replicate tanks were infested two days apart to ensure the window for testing of chalimus and preadult stages was long enough to test all temperatures within the same period. The tanks had a continuous flow-through of filtered and UV-treated seawater from an intake in the adjacent fjord (90 m depth). Fish were kept at a temperature of 14.0°C (± 1) and a salinity of 34‰ in all tanks, which were continuously illuminated (24 h light, 0 h dark) to minimize the disturbance and keep the conditions the same. All fish were continuously fed to satiation (3-4.5 mm pellets, Skretting, Norway). Both fish and lice were inspected visually daily. At the preadult and adult stages all fish were heavily infested with lice. The infestations caused bruising on the host fish, and therefore the number of lice was reduced by manually removing lice with tweezers.

2.1.2 *Caligus elongatus*

Adult *C. elongatus* with egg strings (CESenja F12, genotype 1) was obtained from the University of Bergen (UiB), which provided test animals for copepodids and adults. The test animals were incubated, acclimatized, and tested in the same way as *L. salmonis*. All animals of *C. elongatus* were incubated at 9°C, which is the temperature they were kept at UiB.

2.2 Experimental setup

The temperature dependence of metabolic rate in sea lice was estimated from oxygen consumption measurements conducted at five temperatures: 4, 8, 12, 16, and 20°C. The rationale behind the choice of temperatures was to test the animals' performance at the temperatures the species typically experiences and at their upper and lower tolerance limits (Hamre et al., 2019). All temperatures were acquired by mixing the amount of tempered inlet water into the holding tanks for lice (cooled seawater 1°C, raw water 8°C, and heated seawater 20°C). Due to fluctuations in water temperature, actual temperatures for the respiration tests ranged from 4.2-4.7 for 4°C, 8.1-9.0 for 8°C, 11.6-12.6 for 12°C, 15.1-16.7 for 16°C, and 19.4-20.4 for 20°C.

A total of 1662 animals (7-150 per life stage and temperature) were measured over 33 experimental runs (Table 1). All life stages of *L. salmonis* were tested at all five temperatures. Copepodids of *C. elongatus* were tested at 8°C and adult females at 4 and 8°C. The testing of *C. elongatus* was not performed on all five temperatures as planned due to equipment breakdown, which led to the lice specimens growing too old before replacement parts arrived.

Table 1. Tested life stages, temperatures, and the number of replicates of sea lice in the experiment. *Challenges of keeping the test temperature at 4°C may have introduced inaccuracies in the dataset for nauplius, so this was not included in the results.

	<i>L. salmonis</i>						<i>C. elongatus</i>	
	Nauplius	Copepodid	Chalimus	Preadult	Adult male	Adult female	Copepodid	Adult female
4°C	*	9	14	10	8	9	-	8
8°C	11	15	14	13	14	7	7	10
12°C	15	14	7	11	12	8	-	-
16°C	8	9	10	8	13	9	-	-
20°C	8	12	9	12	8	6	-	-

Acclimation of the experimental animals was done in the incubators, which had mesh at the bottom, inside another container that allowed inlet water to flow through. The temperatures were gradually adjusted two degrees an hour from incubation temperature (9 or 14°C) to the experimental temperature (4, 8, 12, 16, 20°C). The acclimation was performed over 18-24 hours to avoid stress caused by sudden temperature changes. Nauplii were acclimated for 4 hours to avoid potential molting into copepodids.

The micro-respirometry system (Loligo Systems, Viborg, Denmark) consisted of two glass microplates with 24 wells each (Fig. 9 and Fig. 10). Each well had an oxygen sensor spot, scanned by an optical fluorescence O₂ microplate reader. Prior to measuring oxygen consumption, the system was calibrated with filtered oxygen saturated seawater and de-oxygenized distilled water (by adding sodium sulphite). Microplates with 900 µL wells were used for all stages except for the adult stages of *L. salmonis* which needed more space, and 1700 µL wells were used instead. Larvae from each respective temperature and life stage were assigned randomly to wells (Fig. 9). Nine wells contained only seawater to measure background (control) oxygen concentration. Due to the small size of nauplii and copepodids, individuals were pooled in groups of ~10 to get good respiration readings (varying between 6-15 animals due to difficulties counting animals swimming around the different wells). Chalimus had two larvae per well. For the larger stages of lice (preadult and adult), each well contained a single animal, allowing the metabolic rate to be individually monitored for each animal. After inserting animals into the wells, the wells were sealed by sliding a glass plate over the microplate. The microplates were then placed in a water bath with running water to maintain a stable temperature and logged for 3 hours on average, with oxygen concentration recorded every 15 seconds (Fig. 9). The system was covered with a black bag during testing to remove the influence of light on the behavior and subsequent oxygen consumption of the animals.



Figure 9. *Loligo* microplates with animals in the wells (Photo: Tuva Sørgaard).

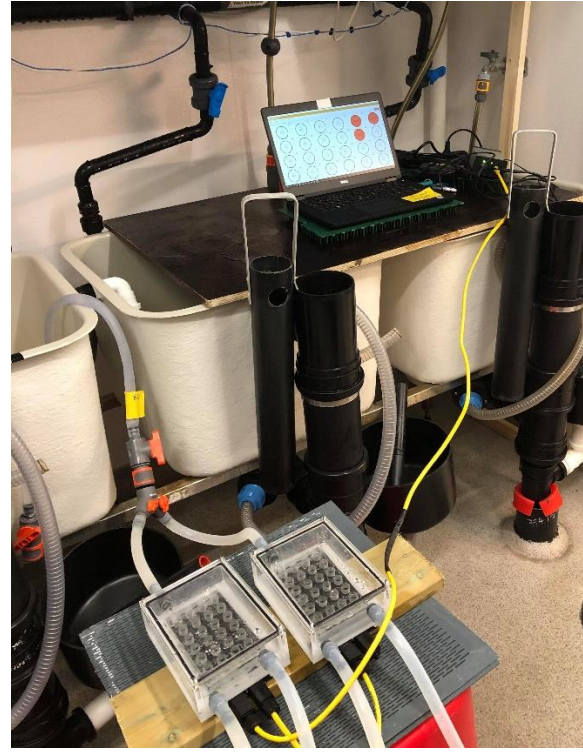


Figure 10. Experimental setup showing inlet water and The *Loligo* Microplate System connected to a computer receiving data (Photo: Tuva Sørgaard).

To measure the body length of preadult and adult stages, digital images of the lice were taken after the respiration measurements. The images were made by using a camera (Toupcam U3CMOS 14 MP 1/2.3”) mounted on the microscope coupled to a computer. Larval total length (TL, mm) was measured in photographs using ImageJ (ImageJ v1.53k 2021).

2.3 Data handling and statistical analyses

To determine the temperature dependence of metabolic rate, oxygen consumption was calculated based on the slope of the linear decline in oxygen concentration, corrected for temperature, salinity, and air pressure. The average measuring time for each respiration run was 3 h, where the longest test interval was 5 h and the shortest was 35 min. For analysis, the first 20 minutes of each run were excluded to avoid measurement fluctuations due to handling stress of the animals. In runs where the temperature had changed drastically during the experiment,

the values were corrected with the use of the control wells. To ensure good data quality, measurements that did not reach the R^2 limit (0.95 for nauplii and copepodids, 0.90 for the attached and moving stages) were discarded. The statistical tests were performed using RStudio v.4.0.2 (R Development Core Team, 2020). Statistical significance was accepted at $p < 0.05$. An analysis of covariance (ANCOVA) from the package car v.3.0-12 (Fox and Weisberg, 2019) was performed to test the interaction between temperature and life stage on oxygen consumption. To test for differences in the oxygen consumption at the different temperatures, a one-way analysis of variance (ANOVA) in the package car v.3.0-12 (Fox and Weisberg, 2019) was used for each life stage. The data were checked for normality using the Shapiro-Wilk test in the package stats v.4.3.0 (R Core Team, 2020) and homogeneity of variance using Levene's test in car v.3.0-12 (Fox and Weisberg, 2019). Based on the assumption that extreme values may indicate active or stressed lice rather than routine metabolism, outliers were identified and removed using the outlierTest function in car v.3.0-12 (Fox and Weisberg, 2019). One extreme value in the dataset of *L. salmonis* adult females tested at 20°C was found by outlierTest but was kept in the analysis due to the small sample size. Tukey multiple comparison test from package stats v.4.3.0 (R Core Team, 2020) was used to identify the significant differences between oxygen consumption means. Where the assumptions for one-way ANOVA were not met a Welch's ANOVA, R package stats v.3.0-12 (R Core Team, 2020), with a Dunnett's T3 test, R package PMCMRplus v.1.9.3 (Pohlert, 2021), as post hoc, or a Kruskal-Wallis test, R package stats v.3.0-12 (R Core Team, 2020), with a Dunn's test, R package FSA v.0.9.3 (Ogle et al., 2022), as post hoc was performed. To test for differences in size (L) between and within life stages of preadult and adults, a regression analysis using the lm function was performed. The ggplot function in R package ggplot2 v.3.3.5 (Wickham, 2016) was used to visualize the raw data.

3.0 Results

3.1 Respiration in *Lepeophtheirus salmonis*

An increase in oxygen consumption was observed with increasing life stages and temperatures (Fig. 11). This effect was significant both for temperature (ANCOVA, $F=18.081$, $p<0.001$) and life stage (ANCOVA, $F=28.947$, $p<0.001$) and the interaction of the two factors (ANCOVA, $F=4.005$, $p=0.001$).

Nauplius had the lowest oxygen consumption at 12°C with 0.93 ± 0.10 nmol ind⁻¹ h⁻¹ (mean±SD, Fig. 12A). The oxygen consumption for the other test temperatures was 1.48 ± 0.29 , 1.87 ± 0.39 , and 2.9 ± 0.4 nmol ind⁻¹ h⁻¹ for 8, 16, and 20°C, respectively. There was a significant difference between oxygen consumption at different temperatures (Welch's ANOVA, $F=73.754$, $p<0.001$). Post hoc test (DunnettT3 test) revealed significant differences between 12 and 8 °C ($p<0.001$), between 16 and 12 °C ($p<0.001$), and between 20 and 8 ($p<0.001$), 12 ($p<0.001$), and 16 °C ($p<0.001$).

In general, copepodids showed an increase in mean oxygen consumption with temperatures of 0.35 ± 0.04 at 4°C, 0.84 ± 0.14 at 8°C, 0.77 ± 0.34 at 12°C, and 2.27 ± 0.49 nmol ind⁻¹ h⁻¹ at 20°C. However, the highest oxygen consumption was recorded at 16°C, with 3.02 ± 0.66 (Fig. 12B). Temperature had a significant effect on oxygen consumption (Welch's ANOVA, $F=106.48$, $p<0.001$). Post hoc test (DunnettT3 test) revealed significant differences between 8 and 4°C ($p<0.001$), 12 and 4°C ($p=0.004$), between 16 and 4°C ($p<0.001$), 8°C ($p<0.001$), 12°C ($p<0.001$), and between 20 and 4°C ($p<0.001$), 8°C ($p<0.001$), and 12°C ($p<0.001$).

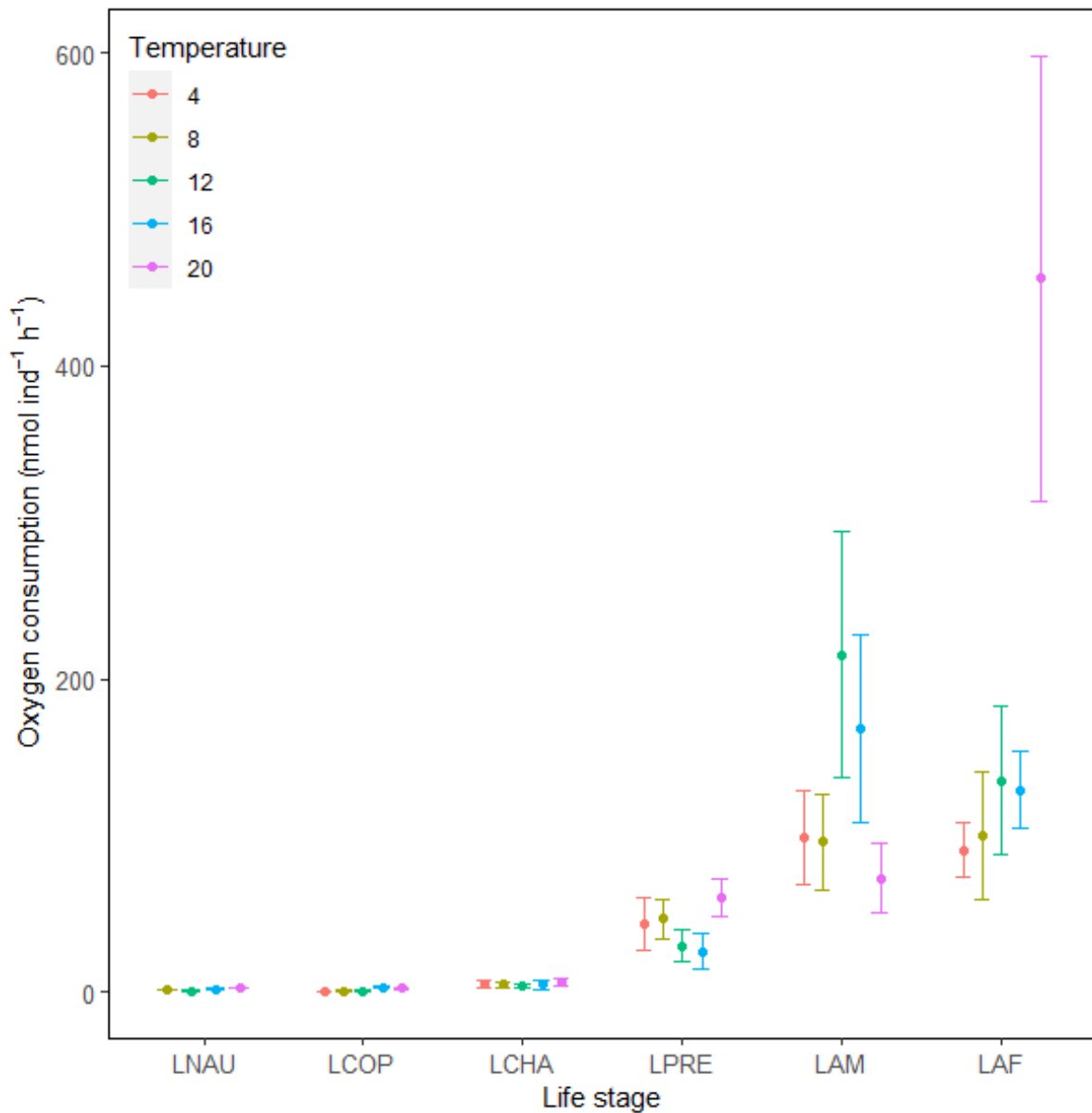


Figure 11. Plot showing an overview of *L. salmonis* life stages and temperatures with the corresponding oxygen consumption. Nauplius (LNAU, $n=42$), copepodid (LCOP, $n=59$), chalimus (LCHA, $n=54$), preadult (LPRE, $n=54$), adult male (LAM, $n=55$), and adult female (LAF, $n=39$). Data presented as mean \pm SD.

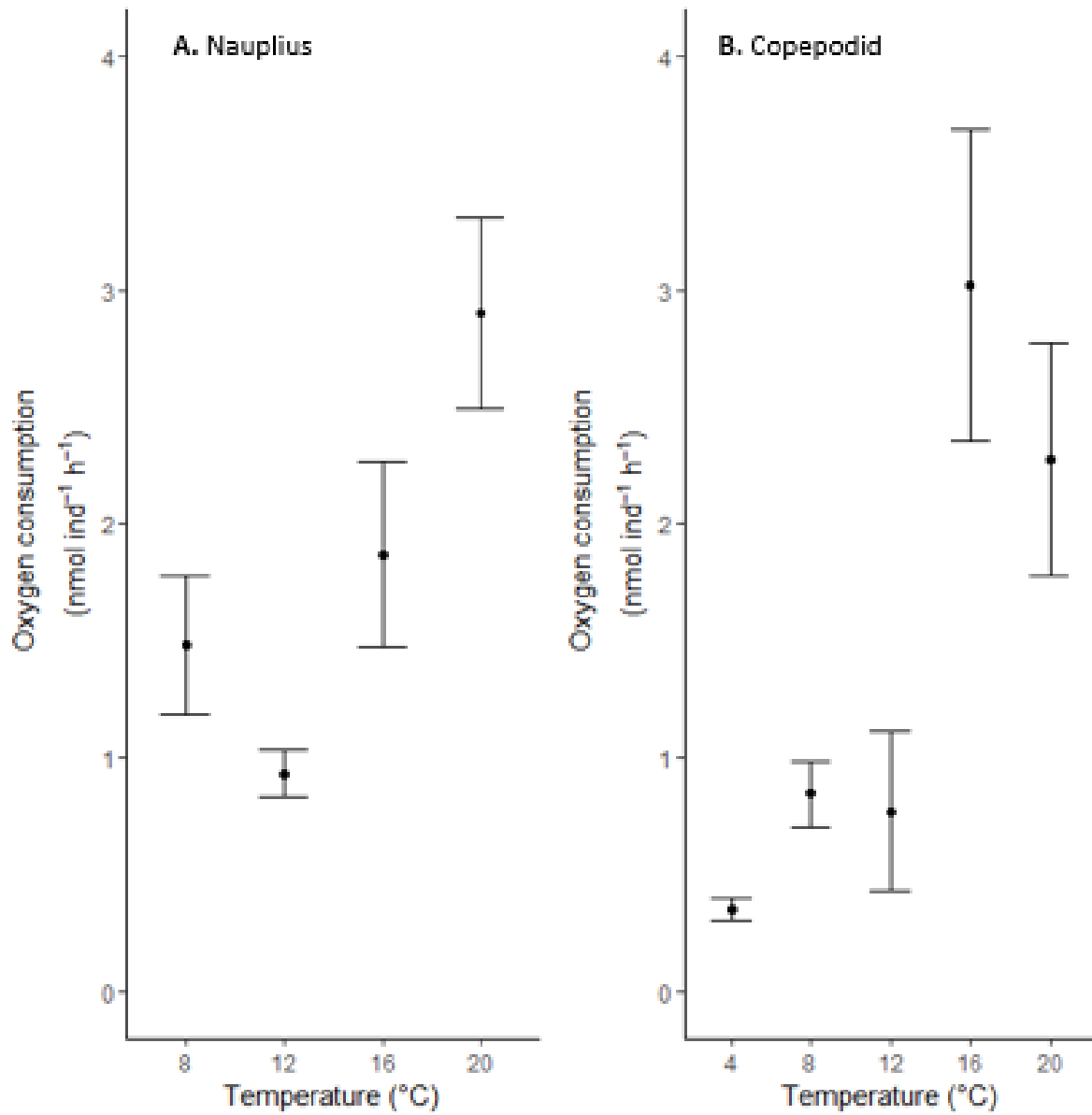


Figure 12. The effect of temperature on oxygen consumption in the planktonic stages of *L. salmonis*. A: nauplii ($n=8-15$), B: copepodids ($n=9-15$). Data presented as mean \pm SD.

Chalimus had the lowest oxygen consumption at 12°C with 3.62 ± 1.02 nmol ind⁻¹ h⁻¹. For the other test temperatures, 4, 8, 16, and 20°C, the chalimii had an oxygen consumption of 5.03 ± 2.01 , 4.69 ± 1.7 , 4.81 ± 2.91 , and 6.47 ± 1.98 nmol ind⁻¹ h⁻¹, respectively (Fig. 13A). No significant differences in oxygen consumption were found for chalimii at the different temperatures tested (Kruskall-Wallis test, chi-squared= 9.355, df= 4, p= 0.053). Even though oxygen consumption increased with temperature, this trend was not statistically significant (p>0.05). A trend was observed with differences in oxygen consumption between 12 and 20°C.

Preadults had an oxygen consumption of 43.43 ± 17.18 , 46.87 ± 12.54 , 29.40 ± 10.41 , 25.96 ± 11.3 , and 60.15 ± 12.23 nmol ind⁻¹ h⁻¹ for 4, 8, 12, 16, and 20°C (Fig. 13B). Temperature had a significant effect on oxygen consumption for preadults (one-way ANOVA, F=12.08, p<0.001). Post hoc test (TukeyHSD) revealed significant differences between 16 and 4°C (p=0.047) and 16 and 8°C (p=0.006), between 12 and 8°C (p=0.014), and between 20 and 4 (p=0.031), 12 (p<0.001), and 16°C (p<0.001).

Adult males had the highest oxygen consumption at 12°C with 215.6 ± 78.07 nmol ind⁻¹ h⁻¹. For the other test temperatures, 4, 8, 16, and 20°C, adult males had an oxygen consumption of 98.89 ± 30.3 , 95.71 ± 30.01 , 168.42 ± 59.9 , and 72.74 ± 22.37 nmol ind⁻¹ h⁻¹, respectively (Fig. 13C). The effect of temperature on oxygen consumption was significant for adult males (Welch's ANOVA, F=12.763, p<0.001). From the post hoc test (DunnettT3 test) significant differences between 12 and 4°C (p=0.002), and 12 and 8°C (p=0.001), between 16 and 4°C (p=0.021), 16 and 8°C (p=0.009), and between 20 and 12°C (p<0.001), and 16°C (p<0.001) were found.

In general, adult females showed an increase in oxygen consumption with temperature of 90.75 ± 17.12 at 4°C, 99.43 ± 40.82 at 8°C, 129 ± 24.88 at 16°C, and 455.63 ± 142.34 at 20°C (Fig. 13D). The oxygen consumption was higher at 12°C with 135.09 ± 47.29 , but not significantly different from 16°C. Temperature had a significant effect on oxygen consumption for adult females (Welch's ANOVA, F=11.903, p<0.001). Significant differences between 16 and 4°C (p=0.017), between 20 and 4 (p=0.010), 8 (p=0.007), 12 (p=0.013), and 16°C (p=0.017) were revealed from the post hoc test (DunnettT3 test).

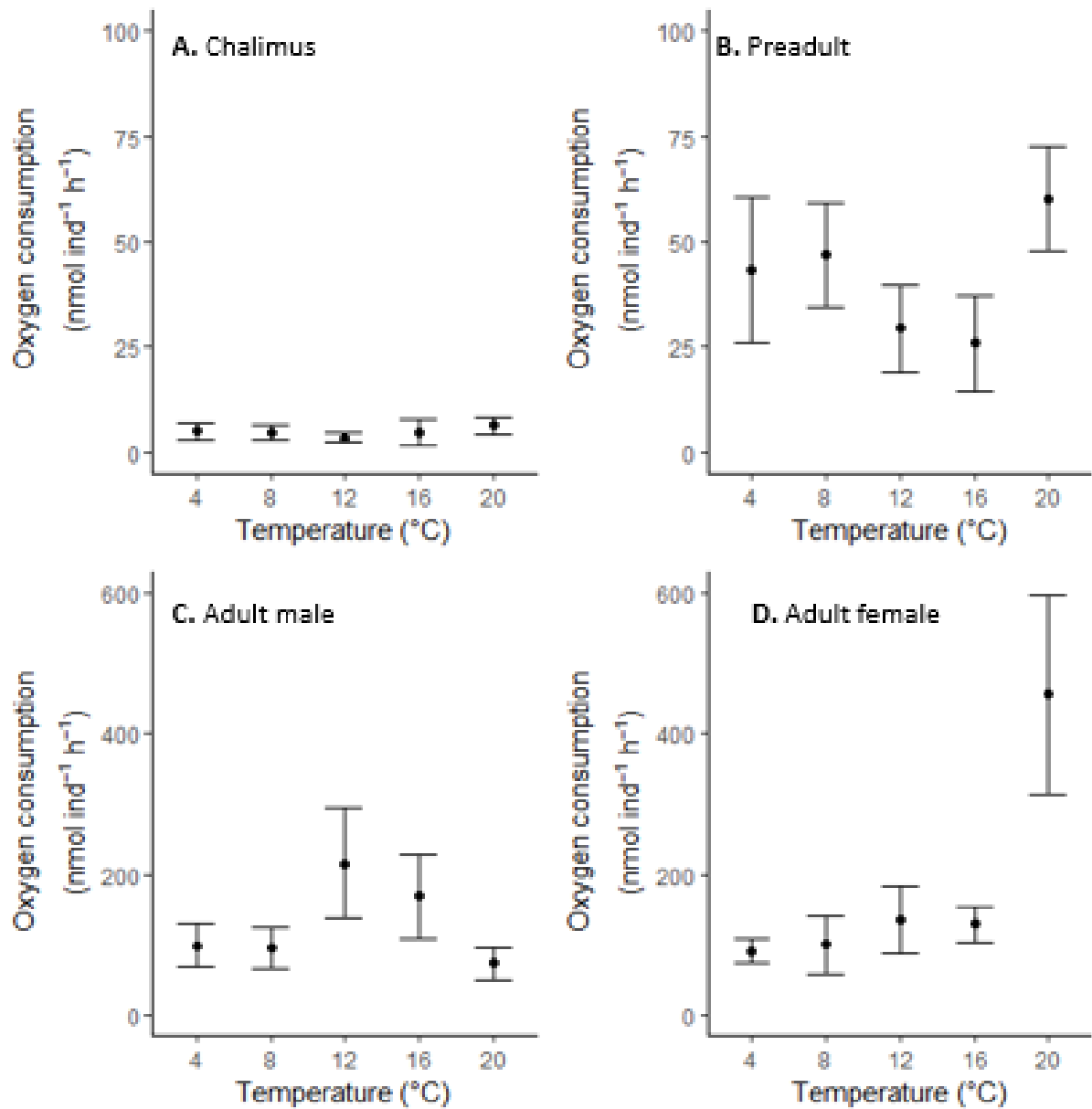


Figure 13. The effect of temperature on oxygen consumption in the attached and mobile stages of *L. salmonis*. A: chalimus ($n=7-14$), B: preadult ($n=8-13$), C: adult male ($n=8-14$), D: adult female ($n=6-9$). Gender was determined based on visual inspection. Scales on the y-axis differ due to large differences in oxygen consumption between stages. Data presented as mean \pm SD.

3.2 Respiration in *Caligus elongatus*

The oxygen consumption of copepodids at 8°C was 0.99 ± 0.24 nmol ind⁻¹ h⁻¹ (Fig. 14A).

Adult females had an oxygen consumption of 65.58 ± 27 nmol ind⁻¹ h⁻¹ at 4°C, and 59.66 ± 20.67 nmol ind⁻¹ h⁻¹ at 8°C. Temperature did not have a significant effect on oxygen consumption in adult females (one-way ANOVA, $F=0.278$, $p=0.605$) (Fig. 14B).

3.3 Comparing respiration in *L. salmonis* and *C. elongatus*

Caligus elongatus copepodids had an oxygen consumption of 0.99 ± 0.24 nmol ind⁻¹ h⁻¹ and *L. salmonis* copepodids 0.84 ± 0.14 nmol ind⁻¹ h⁻¹ at 8°C (Fig. 15A). The oxygen consumption of the copepodids at 8°C did not significantly differ (one-way ANOVA, $F=3.143$, $p=0.091$).

The respiration of *L. salmonis* adult females was 90.75 ± 17.12 nmol ind⁻¹ h⁻¹ at 4°C and 99.43 ± 40.82 nmol ind⁻¹ h⁻¹ at 8°C, while *C. elongatus* adult females had an oxygen consumption of 65.58 ± 27 nmol ind⁻¹ h⁻¹ at 4°C, and 59.66 ± 20.67 nmol ind⁻¹ h⁻¹ at 8°C (Fig. 15B). *Lepeophtheirus salmonis* adult females showed a trend of consuming more oxygen than *C. elongatus* adult females (ANCOVA, $F=3.778$, $p=0.061$).

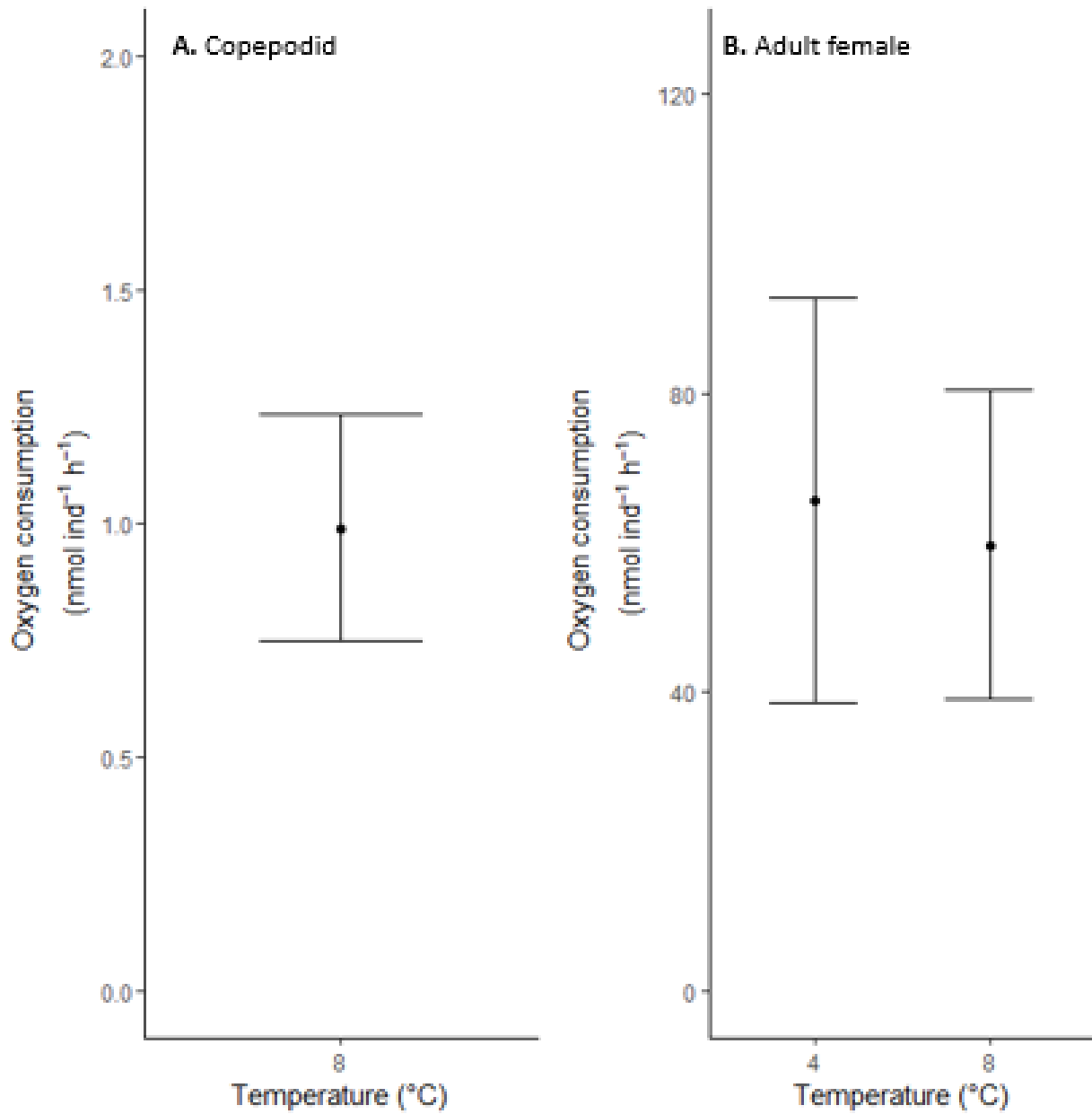


Figure 14. The effect of temperature on oxygen consumption in *C. elongatus*. A: copepodids ($n=7$), B: adult females ($n=8-10$). Scales on the y-axis differ due to large differences in oxygen consumption between stages. Data presented as mean \pm SD.

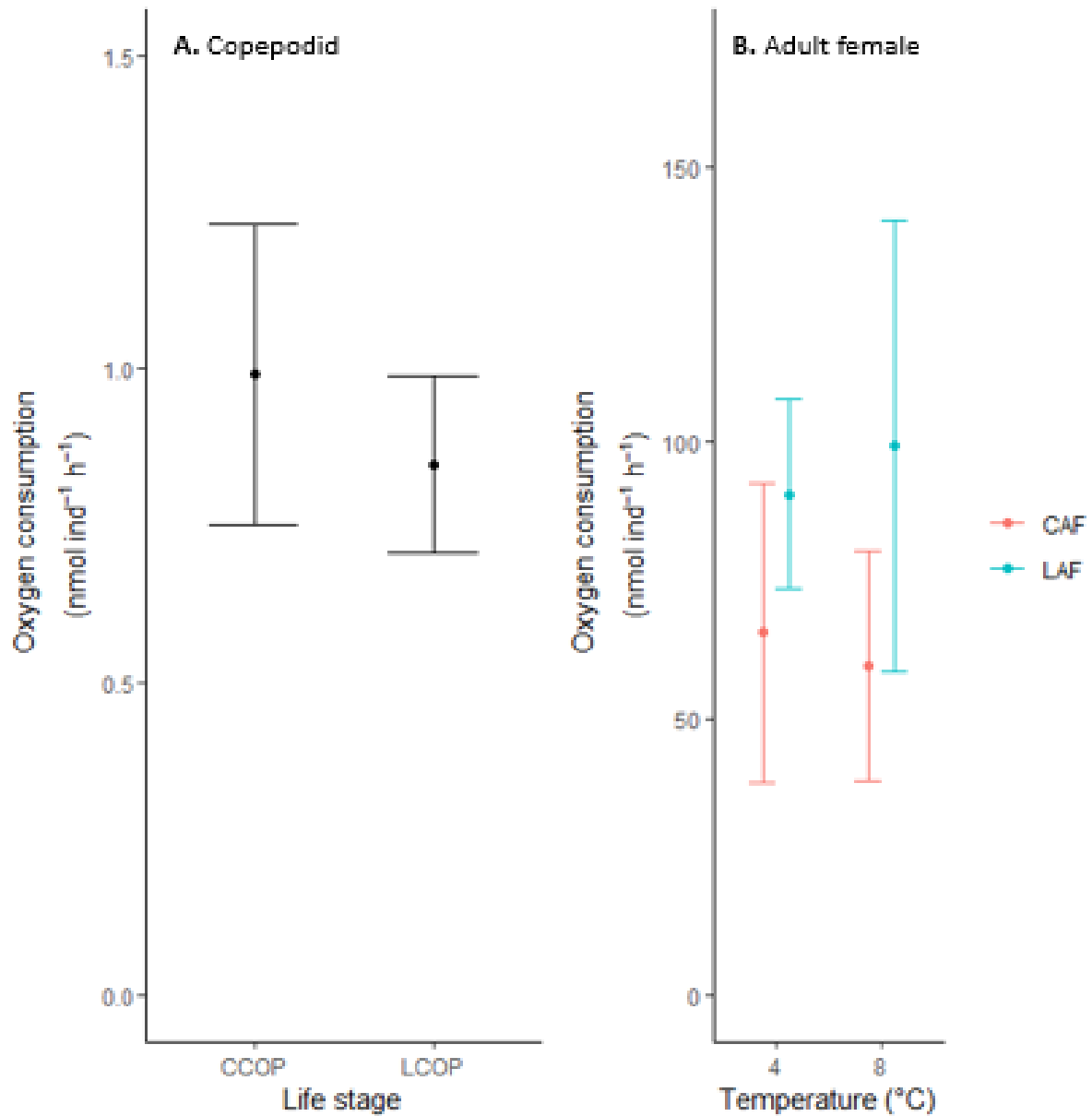


Figure 15. A: The effect of life stage on oxygen consumption at 8°C between *C. elongatus* copepodid (CCOP, $n=7$) and *L. salmonis* copepodid (LCOP, $n=15$). B: The effect of temperature on oxygen consumption between *C. elongatus* adult female (CAF, $n=8-10$) and *L. salmonis* adult female (LAF, $n=7-9$). Scales on the y-axis differ due to large differences in oxygen consumption between stages. Data presented as mean \pm SD.

3.4 Respiration and body length

Body length had a significant effect on oxygen consumption in *L. salmonis* ($p < 0.00001$, Fig. 16). However, body length had no effects on oxygen consumption within the life stages of preadults, adult males, adult females *L. salmonis*, or adult females *C. elongatus* (all $p > 0.05$, Fig. 17).

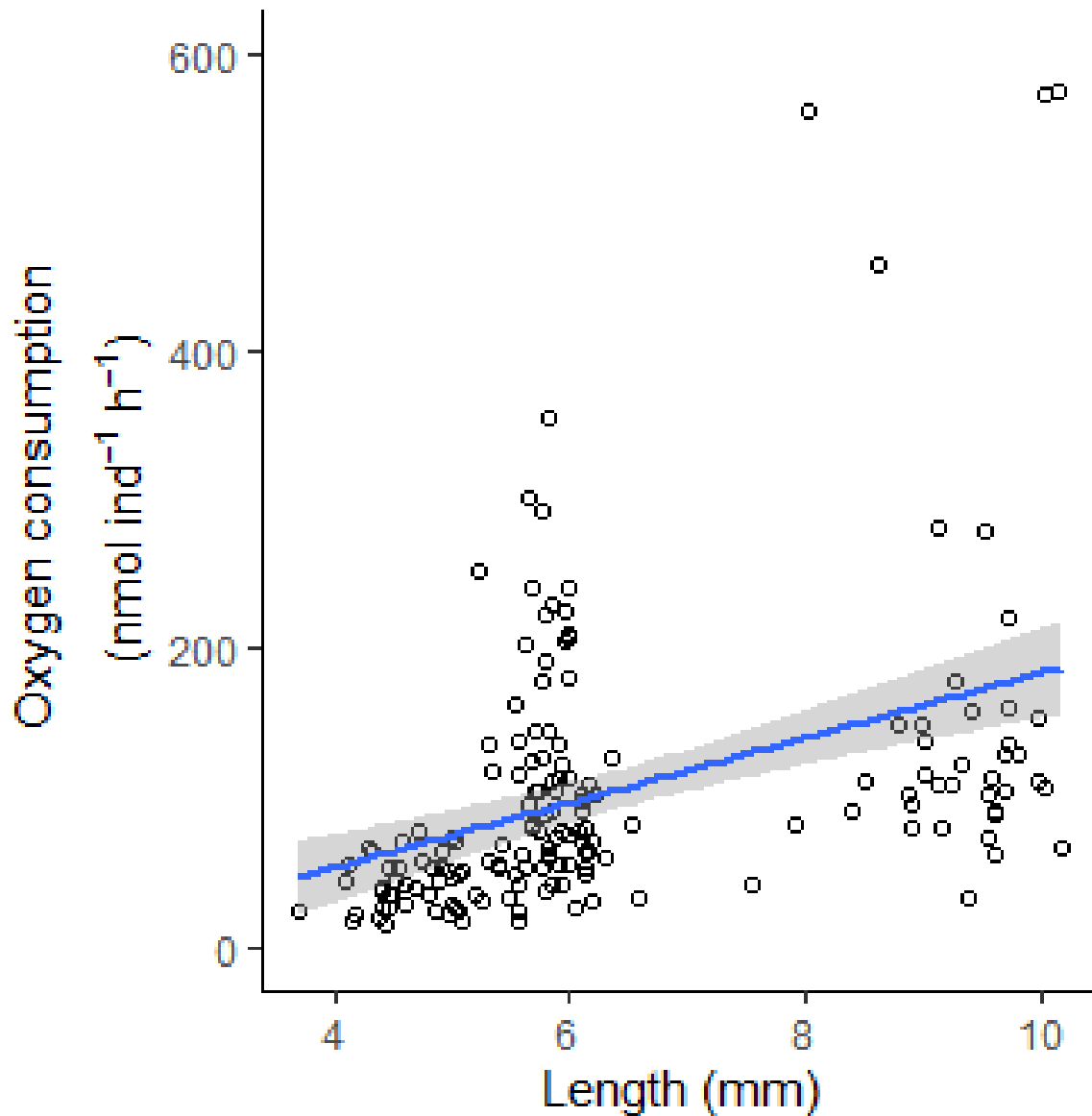


Figure 16. Scatterplot with regression line showing the effect of body length (total length, mm) on oxygen consumption in *L. salmonis* preadult ($n=54$), adult male ($n=53$), and adult female ($n=39$).

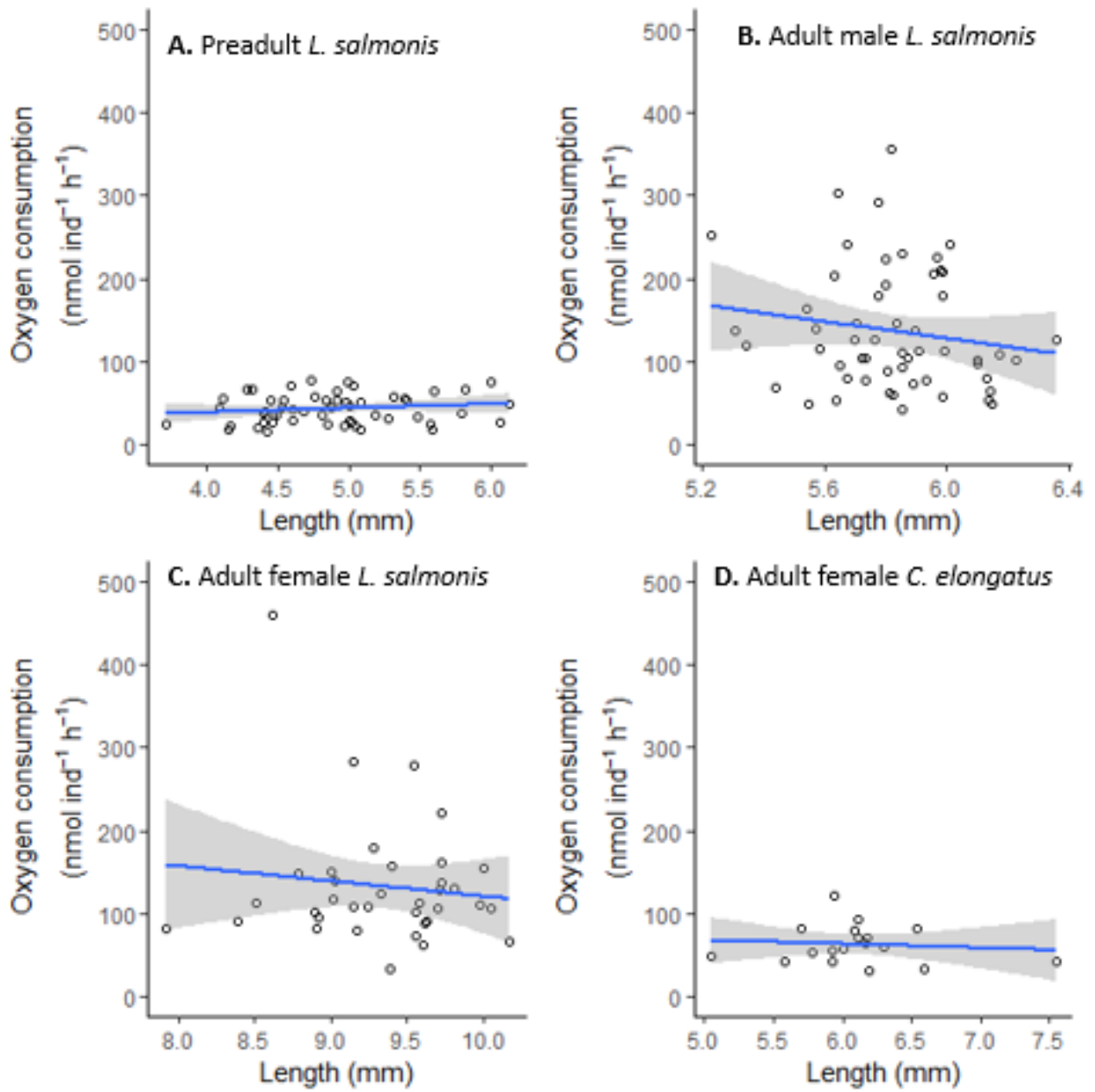


Figure 17. Scatterplot with regression line showing the effect of body length (total length, mm) on oxygen consumption. A: *L. salmonis* preadult (n=54), B: *L. salmonis* adult male (n=53), C: *L. salmonis* adult female (n=39), D: *C. elongatus* adult female (n=18).

4.0 Discussion

In this study, larval respiration rates in *L. salmonis* and *C. elongatus* showed a significant response to temperature exposure only in *L. salmonis*. The low sample size of *C. elongatus* (n=7-10) might explain why there was no significant difference in oxygen consumption between 4 and 8°C in adults of *C. elongatus*. However, many aspects of *L. salmonis*, such as temperature-dependent development, the life stages, and the high fecundity, are similar to other species of sea lice (Groner et al., 2014). Therefore, it is likely that the trends in this study can be extended to other species, including *C. elongatus*. Thus, the rest of the discussion will focus on respiration in *L. salmonis*, but with parallels to *C. elongatus* where present.

4.1 Oxygen consumption in relation to temperature

In general, the oxygen consumption of *L. salmonis* increased with temperature for all stages (Fig. 11). The differences in oxygen consumption between test temperatures were higher in the preadult and adult stages of *L. salmonis*, than the differences in oxygen consumption for nauplii, copepodid and chalimii of *L. salmonis*, which I suggest may be due to the body size and movement of the animals.

The lowest oxygen consumption of nauplii was at 12°C, and the highest was at 20°C (Fig. 12A). However, nauplii tested at 4°C were not included in the analysis due to the highly fluctuating temperature during the experiment. Therefore, it is possible that the lowest oxygen consumption of nauplii would have been at 4°C, the same as for copepodids. Copepodids had the lowest oxygen consumption at 4°C, and the highest oxygen consumption at 16°C (Fig. 12B). The oxygen consumption of copepodids in the present study was close to the findings in Thompson et al. (2019), where copepodids used 0.84 nmol O₂ ind⁻¹ h⁻¹ at 10°C, while in this study the oxygen consumption was 0.77 nmol ind⁻¹ h⁻¹ at 12°C. As temperature strongly affects the duration of planktonic stages for ectothermic marine organisms (O'Connor et al., 2007), this observed increase in oxygen consumption indicates that the window of viable copepodids is short, especially at higher temperatures where the energy reserves of the planktonic stages of sea lice are depleted faster, meaning they will develop faster to the infective copepodid stage but are viable for a shorter period time (Pike and Wadsworth, 1999, Samsing et al., 2016, Gravil, 1996, Montory et al., 2020). At lower temperatures, the metabolism of the lice is lowered and activity is reduced, therefore increasing the longevity of energy reserves (Tucker et al., 2000a, Gravil, 1996). No significant difference in oxygen consumption was found between copepodids of *L. salmonis* and *C. elongatus* tested at 8°C (Fig. 15A).

There was no significant difference in the measured oxygen consumption between temperatures in *L. salmonis* chalimii (Fig. 13A). The non-significant differences in chalimus could be due to these life stages being normally sessile on the host and relatively inactive. In addition, the lice may have been damaged when being removed from the host. Preadults of *L. salmonis* had the lowest oxygen consumption at 16°C and the highest oxygen consumption at 20°C, and while there were statistically significant differences across temperatures indicating a trend, there was considerable overlap between error bars (Fig. 13B).

The oxygen consumption of the adult males was strikingly different from the other life stages (Fig. 13C). Adult males had the highest oxygen consumption at 12°C and the lowest at 20°C. However, the oxygen consumption at 20°C was not significantly different from 4 and 8°C. This may be because of thermal stress at 20°C.

Lepeophtheirus salmonis adult females tested at 20°C had one outlier impacting the result, which was kept in the analysis due to the small sample size. Adult females had the lowest oxygen consumption at 4°C and the highest at 20°C (Fig. 13D). The exceptionally high oxygen consumption at 20°C in adult females contrasted with the low consumption in males at the same temperature. The difference in oxygen consumption between adult females of *L. salmonis* and *C. elongatus* was not significant (Fig. 15B), which was surprising due to the size difference between the species (*L. salmonis* 9.33 ± 0.55 mm, *C. elongatus* 6.09 ± 0.50 mm, mean length \pm SD). However, there was a trend toward significance ($p=0.061$), indicating that a larger sample size could result in a significant difference in oxygen consumption between *L. salmonis* and *C. elongatus*.

4.2 Oxygen consumption in relation to life stage

As animals grow, oxygen demands increase, which is in accordance with the increase in oxygen consumption with stage in this study (Fig. 11). Body length had a significant effect on oxygen consumption in the mobile stages (Fig. 16). There was no significant effect of body length on oxygen consumption within the life stages of preadult and adults (Fig. 17). This is not surprising because crustaceans moult between life stages, and therefore within a population there is negligible difference in body size within a life stage.

The oxygen consumption rate of *L. salmonis* nauplii was similar to that of the copepodids at 8°C, 12°C, and 20°C (Fig. 12). This contrasts with the findings of Thompson et al. (2019), who found that copepodids consumed more oxygen than the naupliar stages. They noted that the high oxygen consumption in the copepodid stage might be related to the increased metabolic

cost of detecting and finding a host. However, neither in the study by Thompson et al. (2019) nor this study was a host available for the copepodid stage. Thompson et al. (2019) noted that the oxygen consumption started to decrease in the copepodid stage after reaching a maximum at 7 days post-hatching. As the copepodid age, the energy reserves become depleted, and the infectivity (Tucker et al., 2000b, Skern-Mauritzen et al., 2020) and swimming activity are consequently reduced (Bron et al., 1993).

The nauplii oxygen consumption was measured close after hatching, where they had a lot of energy reserves and use a lot of the energy for growth. However, the copepodids low energy consumption compared to nauplii could not be explained by the age of the copepodids, as they had only been copepodids for one day before oxygen consumption measurements. Difficulties in counting nauplii and copepodids as they were moving around on the microplates could have given an inaccurate number of animals in each well, increasing variability in the results. However, this counting error would have been random and should not bias the results.

4.3 Optimal temperatures

Johannessen (1977) suggested that the optimal temperature range for *L. salmonis* is 9-11°C, based on development time, egg-bearing period, and hatching time. Tucker et al. (2000a) demonstrated that settlement and survival of *L. salmonis* copepodids were more successful at 12°C than at 7°C. In a study by Hamre et al. (2019), the development from copepodid to adult had an approximately equal duration at 6-21°C. The findings by Hamre et al. (2019) might suggest that the optimal temperature for growth in the attached and mobile stages of *L. salmonis* lies somewhere in this temperature range. In the present study, nauplii and chalimii of *L. salmonis* had the lowest oxygen consumption at 12°C. Preadult and adult female had the lowest oxygen consumption at 16°C and 4°C, with no significant differences to 12°C. These findings might imply that 12°C is close to an optimal temperature for the species, and do not contradict previous studies.

Copepodids of *L. salmonis* showed a rising oxygen consumption with temperature and a decline beyond a certain upper temperature (Fig. 12B). This suggested that around 16°C may be closer to the optimum for this life stage. But the nauplii did not show a decline at 20°C, and neither did chalimii, preadults, or adult females (Fig. 13A, B, and D). However, adult males declined in oxygen consumption beyond 12°C (Fig. 13C). These results suggest that the temperature range of survival for *L. salmonis* is outside what was tested in this study. I would recommend further experiments to test more and higher temperatures to derive a clearer relationship.

This study could not suggest the preferred or optimal temperature for *C. elongatus* due to the few temperatures tested and low sample size. However, other studies have suggested the optimal temperature for *C. elongatus* to be 14°C (Hogans and Trudeau, 1989) and based on this, earlier seasonal peaks and more annual generations of the lice in the northern parts of its distribution are expected as a result of rising sea temperatures (Hemmingsen et al., 2020). An unusually high number of *C. elongatus* has been found in northern Norway (Imslund et al., 2019), where the sea temperatures are normally below 14°C (Barentswatch, 2021). This observation contradicts published evidence on *C. elongatus*, which suggests that the optimal temperature for *C. elongatus* is 14°C and that they produce fewer generations in colder waters (Hemmingsen et al., 2020, Hogans and Trudeau, 1989). Øines and Schram (2008) suggest that the two genotypes of *C. elongatus* might have different temperature ranges, which might explain the observation mentioned above and needs further exploring.

4.4 Consequences of increased mean sea temperature

The frequency of extreme temperatures experienced by ecosystems is expected to increase due to climate changes (Hofmann and Todgham, 2010). In stressful situations, organisms tend to increase their metabolic rate and energy reallocation into maintenance at the expense of growth, development, and reproduction (Montory et al., 2020, Wang et al., 2018). In a study by Dalvin et al. (2020), *L. salmonis* failed to stay on the host fish at the extreme ends of the natural range of the host fish (3 and 24°C), where the lice likely met their temperature limits and strived to feed and persist. Both nauplii, preadult, and adult females had the highest oxygen consumption at 20°C. The same trend was observed in copepodids in this study, where the highest oxygen consumption at 16°C was not significantly different from 20°C. Chalimus showed a trend where the oxygen consumption was lowest at 12°C and highest at 20°C. According to Hamre et al. (2019), the maximum temperature limit for *L. salmonis* development is somewhere between 21 and 24°C. The lice in this master project could not be tested at 24°C due to difficulties maintaining a stable water temperature. However, the upper critical temperature limits for *Calanus finmarchicus*, *Lepeophtheirus pectoralis*, and *Caligus curtus* were suggested to be 20°C, 16°C, and 14°C, respectively (Scott, 1901, Heegaard, 1947, Hirche, 1987). Together, these results suggest that at 20°C *L. salmonis* are close to their maximum temperature tolerance.

An increase in mean sea temperature may lead to an increased abundance of sea lice due to shorter generation times, affecting the distribution of sea lice and hosts and possibly increasing stress for sea lice and hosts (Costello, 2006, Montory et al., 2018, Groner et al., 2016, Montory et al., 2020). A warmer sea temperature will also increase the infestation pressure of *L. salmonis*

from fish farms to wild salmonids (Sandvik et al., 2021). An increase in infestation pressure of sea lice with a factor of 4.4 is expected where the sea temperature increases from 5 to 7°C (Sandvik et al., 2021). The effect of climate warming will play out differently between seasons and geographical areas, and the impact of a 2°C temperature increase may be relatively more significant in areas with low temperatures, depending on absolute temperature limits for the species (Sandvik et al., 2021).

4.5 Methodological challenges

Organisms can compensate with the reallocation of resources within the organism's energy budget or by changes in behavior, making it difficult to determine exact energetic costs (Thompson et al., 2019, Pan et al., 2015, Newell and Branch, 1980). Sea lice nauplii are nonfeeding (lecithotrophic), meaning they cannot compensate for the increased metabolic cost through increased ingestion. Directly measuring changes in consumption rate for the nonfeeding life stages makes for realistic measurements of the changes in metabolism in response to temperature (Thompson et al., 2019). All nauplii, copepodids, chalimii, and preadults of *L. salmonis* in this study were of the same age when tested. However, the adults of *L. salmonis* and *C. elongatus* were kept in incubators between 2 and 6 days before all were tested. This could have resulted in varying energy reserves in the lice, ultimately affecting the respiration measurements.

In this project, the attached and mobile stages (chalimus, preadult, and adult) were tested off the host, and in a laboratory, due to practical limitations of the Loligo oxygen measurement system. Therefore, the exact outcome of an in vivo trial cannot be predicted. It is still interesting to test these stages off the host since *C. elongatus* adults can be live planktonic, where they can reinfest new fish (Øines et al., 2006, Neilson et al., 1987). Adult males and preadult II females of *L. salmonis* can also change hosts when the distance is short, which is thought to increase the possibility of successful mate location (Hull et al., 1998, Ritchie, 1997). The temperatures used in this study are comparable to sea temperatures within the host fish's geographic range and what can be expected in nature around fish farming sites of Norway. However, there were fluctuations in water temperature during respiration measurements which could affect the results. A study by Lyytikäinen and Jobling (1998) indicated that fluctuating thermal conditions increase metabolic rate higher than from data collected at a constant temperature.

Acclimatory abilities allow organisms to tolerate a wider range of temperatures. Ljungfeldt et al. (2017) demonstrated that genetic variation occurs in salmon lice for heat tolerance, so the

results from the current study with lice from various farms in Masfjorden might have given a different result than a study performed with lice from another location. Samsing et al. (2016) suggest that lice from temperate waters can be better adapted to warm temperatures, and Boxaspen and Næss (2000) demonstrated that eggs from cold-adapted (6.8°C) *L. salmonis* hatch and develop to copepodid stages as low as 2°C. These studies show that genetics, origin, and acclimation might affect the response of sea lice to temperature challenges (Samsing et al., 2016, Johannessen, 1975, Ljungfeldt et al., 2017). Therefore, a future study should include lice from a wide geographical area.

Despite some research, the role of temperature on sea lice population dynamics is uncertain (Groner et al., 2014, Saksida et al., 2007). Similar controlled laboratory experiments have documented a strong effect of short-term temperature stress on sea lice, whereas long-term studies on farms do not provide clear temperature effects (Groner et al., 2014, Stien et al., 2005). Several studies have found no detectable effect of ambient temperature on sea louse abundance (Heuch et al., 2002, Revie et al., 2002b, Saksida et al., 2007, Revie et al., 2003), which could imply that the sea temperature exposure in those studies did not have as big an influence on abundance levels of *L. salmonis* as other factors (e.g., lice control treatments) or as previously reported (Tully, 1989, Boxaspen, 1997) or that the temperature variation around the sites of study was not big enough to affect the population levels (Saksida et al., 2007).

The oxygen consumption of *C. elongatus* should be further studied at a broader range of temperatures. For further research on sea lice, there should be the same number of individuals for each temperature group to ensure results that are not affected by sample size. Despite limitations, the results from this study can be used to explain the degree of infestation and predict the responses of sea lice to altered sea temperatures following climate change.

5.0 Conclusion

This is the first time the oxygen consumption of attached and mobile stages of *L. salmonis* has been reported. The current study demonstrates how oxygen consumption of *L. salmonis* increases with body length, life stage, and temperature. However, the variable responses between individuals suggest more replicates, and perhaps more temperatures need to be tested to determine a general relationship.

The absence of significant differences in oxygen consumption in *C. elongatus* can be attributed to only two cold temperatures being tested, and a low sample size. *Lepeophtheirus salmonis* tested at 12°C had the lowest oxygen consumption, suggesting that 12°C may be optimal for the species. At 20°C the species exhibited significantly high oxygen consumption, suggesting that they may have been stressed and at or beyond their upper thermal tolerance. As no other published studies have yet looked into temperature-related performance in the attached and mobile stages of sea lice, this is the first information on optimal temperature investigated from an oxygen consumption perspective. The considerable variability amongst individual animals, suggest that handling stress, pre- or post- moult status, stress response that may lead to increased or decreased metabolic activity, and/or other factors influencing individual variability affect oxygen consumption.

6.0 References

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