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Early development of the ectoparasite *Caligus rogercresseyi* under combined salinity and temperature gradients

Jaime A. Montory[a,*], Juan Pablo Cumillaf[b,g], Victor M. Cubillos[d,e], Kurt Paschke[b,c], Mauricio A. Urbina[f], Paulina Gebauer[a]

[a] Centro i~mar, Universidad De Los Lagos, Casilla 557, Puerto Montt, Chile
[b] Instituto de Acuicultura, Universidad Austral de Chile, Casilla 1327, Puerto Montt, Chile
[c] Centro FONDAP de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Chile
[d] Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile
[e] Laboratorio de Recursos Acuáticos de Calfuco, Facultad de Ciencias, Universidad Austral de Chile, Chile
[f] Departamento de Zoología, Universidad de Concepción, Concepción, Chile
[g] Programa de Doctorado en Ciencias de la Acuicultura, Universidad Austral de Chile, Los Pinos s/n, Balneario Pelluco, Puerto Montt, Chile

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ABSTRACT

One of the pathogens causing the highest economic impacts on the Chilean salmon industry is the ectoparasite copepod *Caligus rogercresseyi*, whose abundance is strongly influenced by environmental variables (e.g. salinity and temperature). Infested fish with sea lice reduces appetite, food-conversion efficiency and increase stress level, which results in decreased growth. The skin wounds, caused by the ectoparasite feeding, leave fish exposed to secondary infections and antiparasitics treatment expenses are high. To evaluate the impact of environmental variables on the early development of *C. rogercresseyi*, egg strings were obtained from mature females (reared in laboratory conditions) and exposed to different combinations of salinity (14, 20, 26 and 32 PSU) and temperature (6, 9, 12, 15 and 18 °C). The hatching success and time, pelagic life time, survival and size of nauplius I, nauplius II and copepodid were quantified. Our results indicate that salinity and temperature have a significant effect on the hatching success of this parasite. Salinities between 26 and 32 PSU result in a hatching success of 100%, whereas lower salinities (14 PSU) reduce hatching success by 60% (at all experimental temperatures) generating an increase in mortality of these early developmental stages. A temperature reduction from 18 °C to 6 °C in culture conditions significantly extended the incubation time of *C. rogercresseyi* by 50%. Specifically, temperature had a higher impact on nauplius I and nauplius II larvae, increasing development times to 50 and 100 h respectively, when temperature was decreased to 6 °C. Although combination of salinity and temperature have a significant effect on hatching time and survival in *C. rogercresseyi*, the combination of these variables had no impact on the size of nauplius I, nauplius II and copepodid stage. Thus, the effects on the survival and pelagic life time of the early stage of *C. rogercresseyi*, might substantially affect the fitness of the species under fluctuating conditions of salinity and temperature.

1. Introduction

Sea lice have become one of the main problems for salmon farming worldwide, threatening the productivity of both salmon and trout farming (Burridge et al., 2014). This ectoparasitic copepod attaches to the skin of its host to feed on its mucus, generating wounds and therefore exposing fish to secondary infections, even leading to death at high infestation levels (Pike and Wadsworth, 1999; Costello, 2009; Valdés-Donoso et al., 2013; Oelckers et al., 2014; Lhorente et al., 2014). Depending on the level of infestation, the fish could have reduced appetite, decrease food-conversion efficiency and increased stress, generating a decrease in growth (Pike and Wadsworth, 1999; Johnson et al., 2004; Costello, 2009; Godwin et al., 2017). This in turn, increases production costs by extending the period to harvest and due to the handling involved in the application of antiparasitic treatments (Bravo, 2003; Johnson et al., 2004; Costello, 2009). Worldwide, economic losses caused by sea lice infections in 2009 were estimated to be as high as 0.19 € kg^{-1} of salmon produced (Costello, 2009) and recently in the Norwegian industry the economic losses for sea lice were estimated to be as high as 0.39 € kg^{-1} of biomass produced (Abolofia et al., 2017).

* Corresponding author.
E-mail address: jaime.montory@ulagos.cl (J.A. Montory).

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In Chile, the main parasite species is *Caligus rogercresseyi* (Boxshall and Bravo, 2000). The life cycle of *C. rogercresseyi* involves 8 stages (González and Carvajal, 2003), three non-feeding (lecithotrophic) free-living larval stages (2 nauplii and 1 copepodid) followed by 5 parasitic stages (4 chalimus and 1 adult) (González and Carvajal, 2003). Sea lice infections in Chile have mainly been controlled by the application of chemical treatments (Agusti et al., 2016). Organophosphates applied by bath treatments (1981–2001) (Bravo et al., 2014); and avermectins (late 1980s), or emamectin benzoate administered in the feed (late 1990s) (Bravo et al., 2008b, 2010). Later, pyrethroids such as deltamethrin (2007) and cypermethrin (2009) were introduced; and in 2010 the chitin synthesis inhibitor diflubenzuron appeared on the market and the organophosphate azamethiphos obtained authorization in 2013 (Helgesen et al., 2014).

As in any organism, early developmental stages of different invertebrates (i.e. larvae) are often considered to be the most vulnerable part of the life cycle (Chaparro et al., 2008; Bodinier et al., 2009). It has been reported that low environmental salinities (< 25 PSU) negatively affect the hatching rates, decrease swimming activity of nauplii stages and also may generate an increased mortalities in sea lice (Bravo et al., 2015a). Furthermore, lower infestation rates have been reported for the copepodid stage of the sea lice *Lepeophtheirus salmonis* at salinities ≤ 24 PSU (Wootten et al., 1982; Johnson and Albright, 1991; Pike and Wadsworth, 1999; Tucker et al., 2000a). On the other hand, temperature alone also affects the physiological performance of an organism, like demonstrated for several marine invertebrate species (Clarke, 1987; Thatje and Hall, 2016; Colpo and Lopez-Greco, 2017). Temperature directly affects growth rates of copepods by accelerating (increased temperature) or retarding (decreasing temperature) their development rate (Escribano et al., 1997). In *Lepeophtheirus salmonis*, temperature has a marked effect on the lasting of the non-infective stages (nauplius I and II), varying between 223.3 h at 5 °C, 87.4 h at 10 °C and 50.0 h at 15 °C (Tully, 1992). Similar results have also been recorded in *C. rogercresseyi* (González and Carvajal, 2003) and *L. salmonis* (Groner et al., 2016), where elevated temperatures shortened the developmental time and life cycle of sea lice. Temperature and salinity have long been recognized as key environmental factors (Kinne, 1967), and since both alone affect the physiology of an animal, it is also important to evaluate their combined impacts (Pankhurst and Munday, 2011). In fact, there is growing evidence showing that many environmental stressors can act synergistically, highlighting the relevance of evaluating their effects in combination (Darling and Cote, 2008; Pankhurst and Munday, 2011; Todgham and Stillman, 2013). In *C. rogercresseyi* early development little is known about the potential effects of some factors in combination (e.g. salinity and temperature), which is the objective in the present study. This way, considering the several larval stages of *C. rogercresseyi* and that environmental factors never act in isolation, the present study aims to evaluate the combined effects of different temperatures and salinities on hatching success, hatching time, pelagic life time, survival and size at each of the free larval stages of *C. rogercresseyi* (nauplius I, nauplius II and copepodid stages). Such approach may increase the accuracy of sea lice dispersal models of future works using this biological model.

2. Material and methods

2.1. Obtaining and maintenance of egg strings

Adults of *C. rogercresseyi* were collected from a salmon farming company at southern Chile (Puerto Montt, Seno Reloncaví, 41.5–43° S), and transported to the Universidad Austral (Puerto Montt, Chile). Once in the lab, adults were kept in aquariums with controlled environmental conditions (filtered seawater at 32 PSU, 12 °C) together with *Salmo salar* to facilitate the infestation process. To minimize any potential environmental impact from collection site, we used the second batch of egg strings generated by females of *C. rogercresseyi* previously acclimated to laboratory conditions (described above). To obtain egg strings, fish were first anesthetized with benzocaine 20% (v/v) and then ovigerous females where carefully detached from the host. Females carrying egg strings at mid-point of development, typically having a brown/black pigmentation (Pike et al., 1993; Schram, 1993), were selected for all experiments. Therefore, all egg strings used where at the same development. Egg strings, were then maintained in plastic aquariums (1 L) with filtered seawater (32 PSU, 12 °C), constant aeration and a photoperiod of 11:13 (light: dark) until the start of the experiments (between 45 and 48 h).

2.2. Effect of salinity and temperature on the hatching time and success

The individual and combined effects of four salinities (14, 20, 26 and 32 PSU) and five temperatures (6, 9, 12, 15 and 18 °C) on the success and time hatching of embryos were evaluated. The photoperiod used during the experiments was identical to that used during maintaining. Each of the 20 treatments (4 salinities × 5 temperatures) comprises nine replicates, each consisting of a single egg string in a 10 mL container. Egg strings were chosen randomly from the pool of egg strings extracted from a pool of females. In all containers, the seawater was changed daily and observations were made twice daily (morning and afternoon) to determine if hatching was occurring. Thus, the hatching time (h) and hatching success (%) of embryos from the eggs strings was determined, and hatching was considered to have occurred when at least 50% of the larvae in each egg string had been released into the water column. This criterion was established as not all larvae necessarily hatched after the string opened.

2.3. Combined effect of salinity and temperature on the survival of free life stages (nauplius I and nauplius II)

Once hatching had occurred in each of the treatments fifty randomly selected larvae (nauplius I) from each treatment were moved into 3 mL containers with temperature and salinity combinations identical to the hatching treatments. Again, there were nine replicates per treatment. Seawater was changed daily and the survival of nauplius I and subsequently nauplius II were checked and recorded twice a day using a dissecting microscope (Euromex NZ 1903-P) at a magnification of 10 ×.

2.4. Combined effect of salinity and temperature on size and development time of free life stages

A second group of ~50 newly hatched of larvae were exposed to the previously described temperature and salinity treatments. In all containers, the seawater was changed daily. The larvae were monitored daily under a microscope in order to estimate the time (h) taken to moult into the nauplius II and then into the copepodid stage. Development time was considered as the time needed for 50% of the larvae of one replicate (~50 larvae) to moult to the next stage in development. Once individuals reached the copepodid stage (infective stage), they were individually maintained under the treatment conditions until death, to determine the duration of the copepodid stage in the different treatments. Copepodids were considered dead when become immobile and were lying on the bottom of the well. Sea lice are non-feeding larvae that solely rely on yolk reserves to survive and starve to death if they are unable to swim to find a host (Tucker et al., 2000b).

Larval stages were recorded using a digital camera (EMU-3 CMOS 10Mp) mounted on an optical microscope (Olympus model BX41) fitted with a 10 × objective. The total length of each larvae was measured using the image analysis software Scion Image Pro (V.4.5). Depending on the number of survivors in each treatment, between 10 and 20 larvae were randomly selected and measured.

2.5. Statistical analysis

The combined effect of salinity and temperature on hatching success and mortality at nauplius I and II stages was evaluated using an analysis of deviance, using a binomial distribution. Hatching time, developmental time of stages nauplius I and II, and time to death at copepodid stage was also evaluated by an analysis of deviance, but using a Weibull distribution. These analyses were used as the residuals did not present equal variances (Kalbfleisch and Prentice, 2002). When analysis of deviance was significant, multiple comparisons were then performed using a Tukey test (packages multicomp) (Bretz et al., 2016). The effects of salinity and temperature on the size at the nauplius I, II and copepodid stages, were evaluated using a two way ANOVA, after checking parametric assumptions (Shapiro-Wilk and Levene's test); a significance level of 0.05 was applied. The analyses were performed in R, version 3.2.4. (R Development Core Team, 2016), using the packages MASS (Venables and Ripley, 2002), survival (Therneau and Grambsch, 2000) and multicomp (Bretz et al., 2016) available the CRAN repository (www.r-project.org/).

3. Results

3.1. Effect of salinity and temperature on the success and hatching time

Both salinity (χ^2 = 48.283; P = 0.0001) and temperature (χ^2 = 11.565; P = 0.020) in isolation had an impact on the hatching success of *C. rogercresseyi* (Fig. 1), but there was no interaction between the two variables (χ^2 = 8.321; P = 0.759). A 100% of hatching success or close to it was observed for treatments with salinities of 26 and 32 PSU, at all temperatures. At 14 PSU, hatching success decreased to 30, 55, 60, 65 and 35% for the experimental temperature of 6, 9, 12, 15 and 18 °C, respectively (Fig. 1).

Hatching time was affected by temperature (χ^2 = 11.622; P = 0.020, Fig. 1), but not by salinity (χ^2 = 2.030; P = 0.566) nor the interaction between salinity and temperature was significative (χ^2 = 12.229; P = 0.427). Hatching time was considerably extended at 6 °C (88.3 ± 18 h; test a posteriori, P < 0.05) compared to the other temperatures (9, 12, 15 and 18 °C) with a mean time of 43.3 ± 10.8 h. There were no differences in hatching time between the temperatures 9, 12, 15 and 18 °C (Fig. 1).

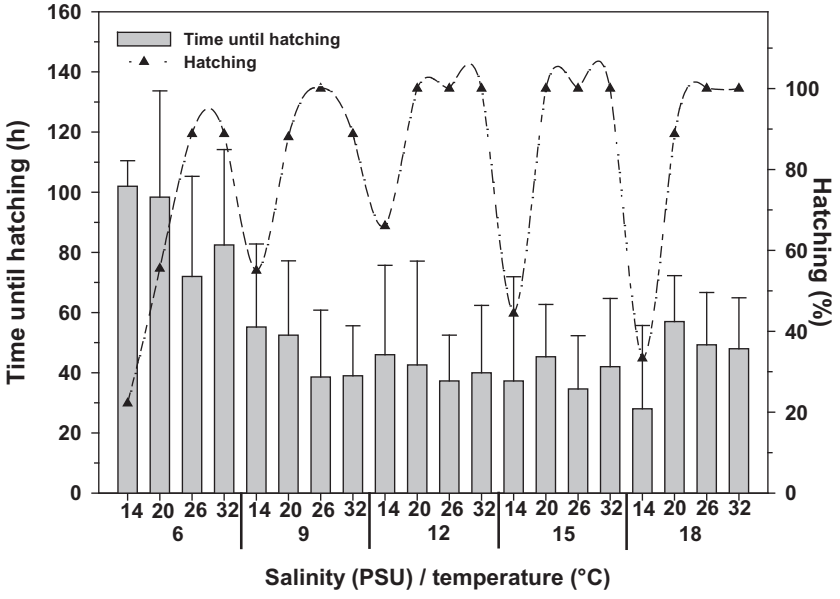

Fig. 1. *Caligus rogercresseyi.* Combined effect of salinity (14, 20, 26 and 32 PSU) and temperature (6, 9, 12, 15 and 18 °C) on hatching time (h) (left axis) and hatching success (%) (right axis) of the egg string. Each bar represents the mean (± SD) of 9 replicates. (N = 180).

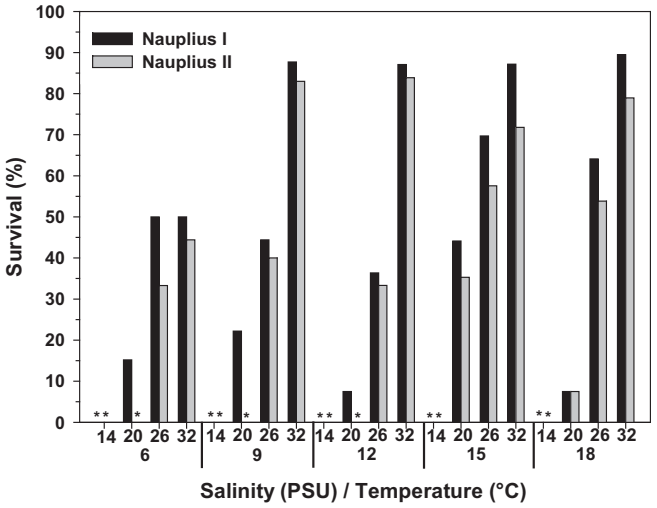

Fig. 2. *Caligus rogercresseyi.* Combined effect of salinity (14, 20, 26 and 32 PSU) and temperature (6, 9, 12, 15 and 18 °C) on survival (%) of nauplius I and nauplius II larvae. * without survivors (N = 135).

3.2. Effect of salinity and temperature on the survival of nauplius I and nauplius II

Survival of nauplius I larvae was significantly affected by the interaction between salinity and temperature (χ^2 = 24.59; P = 0.016; Fig. 2). The highest survival rates were observed in treatments combining temperatures of 9, 12, 15 and 18 °C with a salinity of 32 PSU, where approximately 85% of the larvae survived (Fig. 2). With a salinity of 26 PSU, at all temperatures (6, 9, 12, 15 and 18 °C), the mean survival of the nauplius I larvae was 52 ± 17%. At salinities of 20 and 14 PSU, at all temperatures, mean survival rates of 10 ± 5 and 0% respectively were observed (Fig. 2).

The survival of nauplius II larvae was also significantly affected by the interaction between salinity and temperature (χ^2 = 27.456; P = 0.0001; Fig. 2). With a salinity of 32 PSU combined with temperatures of 9, 12, 15 and 18 °C, approximately 75 ± 6% of all nauplius II larvae survived. In all treatments with a salinity of 26 PSU at all temperatures (6, 9, 12, 15 y 18 °C), the mean survival of nauplius II larvae was 42 ± 15% (test a posteriori; P < 0.05; Fig. 2). For treatments with temperatures of 15 or 18 °C and a salinity of 20 PSU, only a

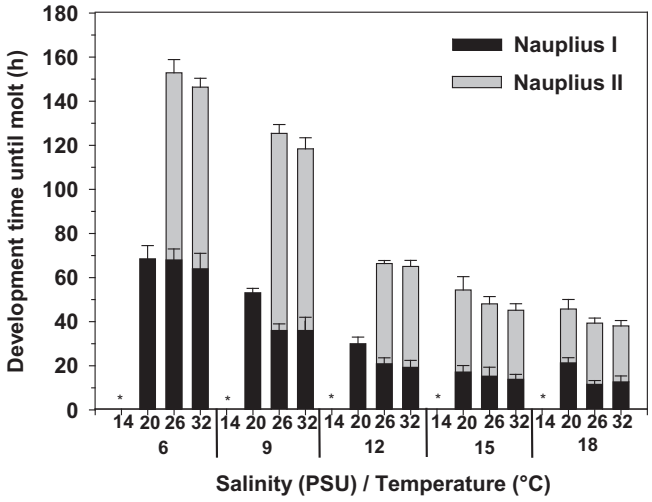

Fig. 3. *Caligus rogercresseyi.* Combined effect of salinity (14, 20, 26 and 32 PSU) and temperature (6, 9, 12, 15 and 18 °C) on development time (h) until moult of nauplius I (black bar) and nauplius II (gray bar). *without survivors. Each bar represents the mean (± SD) of 9 replicates (N = 135).

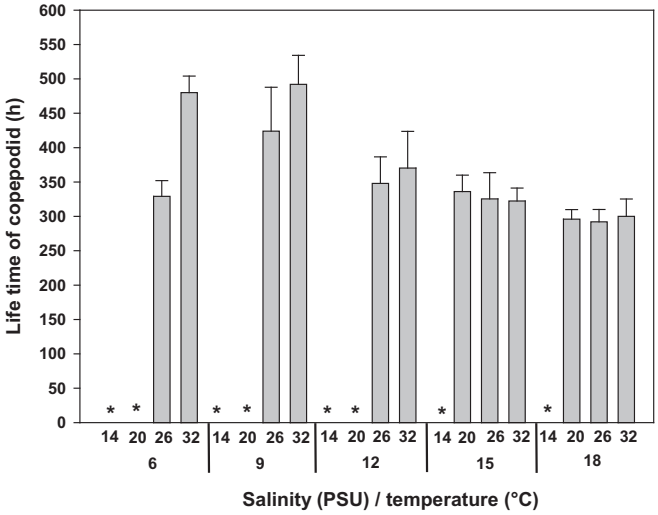

Fig. 4. *Caligus rogercresseyi.* Combined effect of salinity (14, 20, 26 and 32 PSU) and temperature (6, 9, 12, 15 and 18 °C) on free life time (h) of copepodid until the death of the individuals without host. *without survivors. Each bar represents the mean (± SD) of 9 replicates (*N* = 90).

35% and 8% of nauplius II larvae survived respectively. No nauplius II larvae survived in treatments with temperatures of 6, 9 or 12 °C and a salinity of 20 PSU (Fig.2). At 14 PSU, independent of temperature, there is no survival of nauplius II (Fig.2).

3.3. Effect salinity and temperature on size and development time

The development time of the nauplius I larvae were significantly affected by the interaction between temperature and salinity (χ^2 = 28.958; *P* = 0.0003; Fig. 3). In treatments with a temperature of 18 °C and a salinity of 26 or 32 PSU, the mean development time from nauplius I to nauplius II larvae were 11.5 ± 1.7 and 12.5 ± 2.6 h, respectively (Fig. 3). On the other hand, moulting time increased by 40% when nauplius I were exposed to a combination of 18 °C and 20 PSU (21.2 ± 2.3 h). Similar results were recorded in nauplius I exposed to a combination of 9, 12 and 15 °C and 20, 26 and 32 PSU (a Posteriori test; *P* < 0.05; Fig. 3). Only at 6 °C, moulting time from nauplius I to nauplius II was not significantly affected by 20, 26 and 32 PSU salinities, with developmental times of 68.5 ± 6 h, 68 ± 5 h and 64 ± 7 h, respectively (a Posteriori test; *P* > 0.05; Fig. 3).

For the nauplius II developmental stage, the combined effects between salinity and temperature did not generate significant differences (χ^2 = 2.002; *P* = 0.735) in the time required to moult to the copepodid stage. However, temperature (χ^2 = 140.431; *P* = 0.00001) and salinity (χ^2 = 4.948; *P* = 0.026) independently had significant impacts on the development time of the nauplius II stage. Treatments with a temperature of 6 °C and salinities of 26 or 32 PSU, showed the longest developmental times to moult to the copepodid stage, with mean values of 152.5 ± 5 and 148 ± 7 h, respectively (Fig. 3). Conversely, in treatments with a temperature of 18 °C and salinities of 26 or 32 PSU, a significant decrease in the mean developmental time to moult to the copepodid stage was observed, with mean values of 38.9 ± 2.2 and 37.6 ± 2.4 h, respectively (Fig. 3).

Analysis of developmental times in treatments with the combinations of temperatures 15 or 18 °C and a salinity of 20 PSU, were not considered because of the lack of survivors in these treatments (5 and 3 survivors in each stage, respectively).

The survival time of the copepodid stage in the absence of a host varied significantly (χ^2 = 46.159; *P* = 0.0002; Fig. 4) with the impact combined between salinity and temperature. The longest survival time of the copepodid developmental stage was recorded at 6 °C and 9 °C, with a salinity of 32 PSU, with mean survival times of 480 ± 24 and 492 ± 42.2 h, respectively (Fig. 4). At 18 °C survival times decreased, to 296 ± 13.8 h, 292 ± 18 h and 300 ± 25.1 h when animals were exposed to salinities of 20, 26 or 32 PSU, respectively (Fig. 4).

The interactions between temperature and salinity did not significantly affect the sizes of the different stages, nauplius I, nauplius II and copepodid, (2-way ANOVA $F_{(12,71)}$ = 1.42, *P* = 0.175; $F_{(8,60)}$ = 0.55, *P* = 0.875 and $F_{(8,58)}$ = 2.08, *P* = 0.094, respectively). The mean sizes of nauplius I, nauplius II and copepodid for all experimental combinations of salinity and temperature were 387 ± 16.3 µm, 443.4 ± 22.3 µm and 627.8 ± 24.9 µm, respectively (Fig. 5).

4. Discussion

Temperature and salinity are two environmental factors which may have a profound effect on the survival, hatching, rate of development of larvae and the distribution of marine invertebrates (Baylon and Suzuki, 2007; Brazenor and Hutson, 2013). Salinity is known to exert a strong influence on larvae survival in crustacean species, while temperature

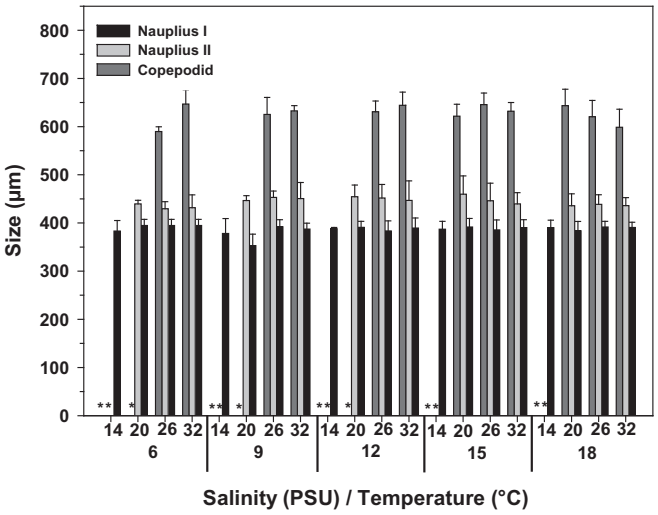

Fig. 5. *Caligus rogercresseyi.* Combined effect of salinity (14, 20, 26 and 32 PSU) and temperature (6, 9, 12, 15 and 18 °C) on size of the free life stages (nauplius I, II and Copepodid). * without survivors. Each bar represents the mean (± SD) of 9 replicates (N = 90).

exerts a weak effect on survival, but a strong influence on the frequency of moulting and the rate of development (Baylon and Suzuki, 2007; Brazenor and Hutson, 2013). The present study is the first report that evaluated the synergistic effects of salinity and temperature on the early development and tolerance of the free living stages (pre-infestation) of the ectoparasite *C. rogercresseyi*. The egg strings of *C. rogercresseyi* exposed to low temperatures (6 and 9 °C), independent of salinity, registered a significant delay in hatching times which suggests that during the cold months of the year, larvae would increase the development time inside of egg strings carried by the female. The development time of larvae was negatively correlated with environmental temperature, which agrees with previous observations in other ectoparasites such as *Salmincola edwarsii* (Poulin et al., 1990), where exposure to higher temperatures (15 and 18 °C) resulted in shortened developmental times of embryos by 13%. In addition, a decrease in the culture water temperature of the ectoparasite *L. salmonis* from 15 to 5 °C, increased in threefold the mean hatching time of the nauplius I stage (Johnson and Albright, 1991).

Larvae of the ectoparasite *Lernanthropus latis,* successfully hatched in a wide range of temperatures (22–36 °C) and salinities (11–35 PSU) (Brazenor and Hutson, 2013). Our results also showed a plasticity in *C. rogercresseyi* hatching that allows this species to succeed hatching in a wide range and combinations of temperature (6–18 °C) and salinity (20–32 PSU). Whether or not the embryos are just tolerant to such variations or the walls of the egg strings confer some buffer against external conditions is still unknown. However, during situations of chemical stress (antiparasitic compounds), hatching success in *C. rogercresseyi* has been reported to decrease only to 50%, and the hatched larvae were not able to complete their development (Bravo et al., 2015b).

It is well known that early developmental stages are prone to the effects of environmental fluctuations, which reduce survival (Verween et al., 2007; Deschaseaux et al., 2011; Zhang et al., 2014; Samsing et al., 2016). In the present research, elevated mortalies (100%) were observed when nauplius I stage were exposed to salinities of 14 PSU and > 50% mortality of nauplius II were also observed at salinities ≤ 20 PSU. In some areas where salmon farms are located in Chile (Seno Reloncaví) environmental salinity can fluctuate between 22 and 32 PSU (Barria et al., 2012), and it has been observed that salinities < 25 PSU reduce the parasite load of *C. rogercresseyi* per fish by 71% (Bravo et al., 2014). A previous study carried out by Bravo et al. (2008a) found that the survival of adults of *C. rogercresseyi* can fluctuate between 100% to 20% after they have been exposed to 20 PSU for 24 h. The authors also indicate that the early developmental stages (e.g. copepodid stage) did not survive at the same salinity levels at 10 °C (Bravo et al., 2015a). The above response was similar to our results, but under conditions of chronic exposure, where at 15 °C a survival of only 36% was obtained up to the copepodid stage at the same salinity (20 PSU). Thus, *C. rogercresseyi* is not tolerant to low salinities (< 20 PSU), likely because reduced salinity affects internal solute concentrations, negatively impacting the efficiency of the physiological processes and its subsequent survival (Kinne, 1967). Similar results have been recorded in other species of marine ectoparasites, for example, Cressey and Collette (1971) observed that the ectoparasite *Lernanthropus tylosuri* infects only fish species that avoid estuarine areas. In addition, Bricknell et al. (2006) identified that survival of the copepodid stage of the sea lice *L. salmonis* was strongly compromised at salinity levels < 25 PSU. Similarly, Heuch et al. (2002) identified negative effects on the survival of copepodid stage of *L. salmonis* and *Caligus elongatus* exposed to low salinity levels (< 20 psu). Changes in environmental salinity cause respective changes in the salinity of internal body fluids.

The planktonic phase of marine invertebrates is an important factor in ecological dispersion models, which are strongly influenced by environmental variables (Jackson and Strathmann, 1981; Amundrud and Murray, 2009; Rittenhouse et al., 2016). Samsing et al. (2017) reported that seasonal variations in lice development times, oceanographic processes and the topological arrangement of salmon farms directly affect sea lice dispersal patterns. For example, it has been identified that population connectivity of sea lice increases between salmon farms as a result of longer maturation times (pre-competency period) and increased pelagic larval duration due to colder water temperatures in winter (Skagseth et al., 2011; Samsing et al., 2017). Thus, the increase in the duration of the pelagic stage of the sea lice larvae may favor or increase both genetic flow (Jablonski, 1986; Stien et al., 2005; Treml et al., 2008; Epifanio and Cohen, 2016; Murray and Salama, 2016; Samsing et al., 2017). However, the latter also increases the vulnerability of larvae to planktonic predators and potentially adverse abiotic factors for longer periods (Pechenik, 1999; Stien et al., 2005). Thus, it is complex to predict the effects at the whole population level this extended pelagic stages may have. In the present investigation the development times of nauplius I and nauplius II larvae of the ectoparasite *C. rogercresseyi*, increased significantly when the temperature decreased at all experimental salinities, a threefold in development time when comparing the extremes of the temperatures used (6 and 18 °C). González and Carvajal (2003) also recorded a decrease in the generation times of *C. rogercresseyi* that were associated with an increase in temperature. They found that a reduction in the developmental time by 50% from nauplius I to copepodid stage was directly associated with an increase in temperature from 11.9 to 16.5 °C (González and Carvajal, 2003). In addition, in the ectoparasites *L. salmonis* and *C. elongatus*, there were also increases in the time to moult between the different stages of development, when the temperature of the water decreased (Wootten et al., 1982; Stien et al., 2005; Groner et al., 2016; Samsing et al., 2016). Thus in Chile, a greater connectivity and infestation of sea lice among the salmon farms, due to the longer pelagic life of the larvae, could be expected during the winter (colder sea water). This highlights how understanding the drivers of sea lice connectivity can enable the design of management strategies to reduce lice transmission and eventually reduce gene flow, slowing down the evolution of drug resistance (Samsing et al., 2017).

An increase in energy costs has been described for larvae exposed to higher temperatures and lower salinities, mainly due to an increase in metabolic rates during exposure to stressful conditions (Johns, 1982; Rasmussen and Tande, 1995; Giménez and Torres, 2002; Castejon et al., 2015), which can negatively affect the stored energy for the growth and survival of the organisms (Giménez and Torres, 2002; Tucker et al., 2000b). In the present investigation, the copepodid (the infective stage) exposed to higher temperatures (18 °C) decreased their free-living time by 33% compared to the same developmental stage exposed to temperatures of 6 and 9 °C. In terms of energy storage, the free living larvae of *C. rogercresseyi* are non-feeding, with the consequence that their development advances there is a reduction in their energy reserves (e.g. lipids, protein and fatty acids) (Kattner et al., 2003; Tucker et al., 2000b), limiting the duration of the pelagic stage. Therefore, the non-feeding larvae of the ectoparasite *C. rogercresseyi* exposed to higher temperatures might accelerates the consumption of their energetic reserves as a consequence of their metabolic demands, with a negative impact on the time they can remain as free-living organisms in the water column (Pappalardo and Fernandez, 2014; Thatje and Hall, 2016).

Although it is well known that salinity and temperature could impact the final size of a variety of marine invertebrate larvae (Johns, 1982; Baylon and Suzuki, 2007; Faleiro et al., 2012), our results indicated that this is not the case for all free living stages of *C. rogercresseyi*. Previous studies carried out in the sea lice *Lepeophtheirus salmonis* and *C. rogercresseyi* reported that bigger adult sizes were reached when they were reared at low temperatures during the whole developmental (Bravo et al., 2013; Samsing et al., 2016). Considering the above mentioned, it is possible that temperature modulate final size in the later stages of *C. rogercresseyi*, but not in the earlier free living stages (as shown in the present study), this needs to be further confirmed.

In summary, the prolonged exposure of egg strings of *C. rogercresseyi*

to a combination of different salinities and temperatures had a significant impact on the hatching success and development time of the encapsulated embryos. Additionally, after larval hatching, we observed a significant effect of salinity, especially on the survival rates of individuals, whereas temperature was the main factor that impacted the development time of free-living larvae. Neither, salinity nor temperature had a significant effect on the size of the early developmental stages of *C. rogercresseyi*.

Sea surface temperatures have been rapidly increasing at a rate 0.11 °C per decade since at least 1971 (IPCC, 2013). This increase in temperature has altered precipitation patterns and increased the rate of glacial melting, causing salinities to decrease in high latitude waters and coastal zones (IPCC, 2013). Thus, the effects of salinity and temperature that are illustrated in this study could have numerous implications for the management of farmed and wild salmonids, for example, the salinity had an important effect on the survival of the larvae of *C. rogercresseyi*, which has been previously observed in adults of *C. rogercresseyi* (Bravo et al., 2008a, 2014, 2015a). The sensitivity shown by *C. rogercresseyi* in this research to reductions in salinity could be a viable alternative for control, especially through fresh water baths. In many areas where salmon are farmed, knowledge of winter precipitation conditions may allow managers to forecast the impact that freshwater runoff will have in controlling sea louse populations. Similarly, the placement of farms in areas affected by riverine input may help to control sea louse infestations if surface salinity is sufficiently reduced to have an effect (Rees et al., 2015). Nevertheless, the adaptive consequence of genetic variation in salinity tolerance has been documented before in marine copepods (Ljungfeldt et al., 2017). The copepod *Eurytemora affinis* has made the transition from marine to freshwater habitat relatively rapid, demonstrating a large shift in the ability to osmoregulate (Lee et al., 2012). The rapid adaptation to new environments could suggest the pre-existence of genetic variation for salinity tolerance (Ljungfeldt et al., 2017). Therefore, the use of alternative treatments (freshwater) may exert a selective pressure on the sea lice, driving it to decreased sensitivity. This needs to be considered when implementing integrated management practices for control of this parasite (Ljungfeldt et al., 2017).

On the other hand, the characteristics of the life cycle of *C. rogercresseyi* that are dependent on environmental temperature (Bravo, 2010), have important implications for the control of parasites in the marine farms. It is very probable that increases in mean sea temperature, resulting from climate change, may lead to an increase in the abundance of this ectoparasite as a consequence of shorter generational times, where, also one alteration in the reproductive strategy it can exist a consequence of fluctuations in environmental parameters, for example, in *C. rogercresseyi* longer egg strings and greater number of eggs per string are produced in winter at a temperature around 10 °C compared with summer temperatures around 15 °C (Bravo et al., 2009; Bravo, 2010). The documented responses to temperature and salinity conditions show that in estuaries, low salinities may limit population growth, while in oceanic conditions, low temperatures may reduce population growth but can increased connectivity between salmon farms, due to the longer pelagic life of the larvae (Samsing et al., 2017). Marine and summer conditions may provide a more favorable environment for sea louse population growth as a result of increased temperatures, which promote faster development and salinities that are not low enough to impact survival (Groner et al., 2016).

Future research should be carried out aimed at evaluating the potential impacts of environmental variables such as salinity and temperature, on the infestation capacity of the copepodid of *C. rogercresseyi* on its host and its subsequent development and fitness to elucidate potential latent and carry over effects.

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