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Life cycle of *Caligus rogercresseyi*, (Copepoda: Caligidae) parasite of Chilean reared salmonids

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Abstract

Caligus rogercresseyi, [Contrib. Zool. 69 (2000) 137] is the only caligid known to affect the salmon industry in Southern Chile. Economic losses due to reduced fish quality, cost of chemical treatment and outbreaks of other diseases such as the Piscirickettsiosis occur. The life cycle of *C. rogercresseyi* is described in rainbow trout reared in seawater tanks from observations made under natural conditions of light and temperature between January 1997 and April 1998. Fish were infected with laboratory-cultured larvae obtained from ovigerous females. Rainbow trout were periodically slaughtered for parasite collection and identification. *C. rogercresseyi* life cycle includes the following stages: two nauplius, one copepodid, four chalimus and the adult. No preadult stage was observed. Timing of the different stages of development was directly dependent on water temperature. The maturation of the eggs or the time for a complete life cycle took place at 45 days in July at 10.3 °C, 31–32 days in April at 12.4 and 12.8 °C, respectively, and at 26 days in November at 15.2 °C. In January, at 16.7 °C, only the appearance of first eggs were observed at 18 days. A simple degree–day (dd) model is proposed for each developmental stage between 4 and 17 °C, where the development rate is a linear function of the average temperature of water. Using this degree–day model, the proportion of fourth stage chalimus was maximum at 172 dd of effective temperature, adult males at 193 degree–days, adult females at 208 dd. The minimum temperature threshold is at 4.2 °C where there is no development of the parasite. The appearance of first eggs occurred at 231 dd and the first pigmented eggs at 277 dd. The temperature-independent degree–

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days value allowed to predict the timing of *C. rogercresseyi* life cycle at any temperature within the evaluated range.

Keywords: Sea lice; Salmon industry; Chile; Generation time; Developmental stages; Degree–days; Minimum temperature

1. Introduction

Caligus rogercresseyi Boxshall and Bravo (2000) is the dominant sea lice parasite affecting the salmon and trout industry in Southern Chile. At the beginning of salmon production in the country, Reyes and Bravo (1983) reported the presence of *Caligus teres* Wilson (1905) in coho salmon (*Oncorhynchus kisutch* Walbaum). Another species, initially identified as *Caligus flexispina*, but now identified as a new species, *C. rogercresseyi* by Boxshall and Bravo (2000) was also reported in 1992 (González and Carvajal, 1994) on rainbow trout *Oncorhynchus mykiss* (Walbaum).

The parasite was transmitted to the farmed fish by the native rock cod *Eleginops maclovinus* and *Odonthestes regia* (Carvajal et al. 1998). These were the most common wild hosts of *C. rogercresseyi*, *C. teres*, *Caligus cheilodactylus* Krøyer (1863) and *Lepeophtheirus mugiloidis* (Villalba and Durán, 1985). The species identified in most rainbow trout and Atlantic salmon in affected farms is *C. rogercresseyi* present today in a proportion of 99% in marine netpens.

Economic losses due to the parasite have led to the control of the caligid with chemicals, despite a lack of a thorough knowledge of its basic biology. The present paper describes the developmental stages of *C. rogercresseyi* and the duration of its life cycle at different times of the year on the rainbow trout held in tanks.

2. Material and methods

2.1. Life cycle

Ovigerous females of *C. rogercresseyi* with pigmented eggs were collected from netpen-reared rainbow trout and Atlantic salmon near Puerto Montt and Chiloé in January, April, July and October, 1997 and March 1998. They were transported in seawater to the laboratory, in beakers packaged in isothermic box. Hatched eggs were kept in 1-l beakers at room temperature with gentle aeration and daily changes of filtered seawater (1 μm, 29–31 ‰). The temperature incubation of larvae was 16.5 °C in average (range: 14.6–19.3 °C) in January 1997, 13.2 °C (11–14.9 °C) in April 1997, 9.6 °C (8.7–10.6 °C) in July 1997, 11.9 °C (7.5–14.5 °C) in November 1997, and 17.9 °C (13.5–19.5 °C) in March 1998.

Females with empty sacs were separated on the second day and were fixed in 75% ethanol for taxonomic identification. The planktonic larvae were counted between days 5

and 7, when 100% had reached the copepodid stage. The volume of cultures was reduced to 95 ml for counting by filtration. Beakers were shaken for homogenization and 5-ml aliquots were prepared and counted in tissue culture chambers. In the five experiments on life cycle studies, copepodids were placed in a 500-l tank containing 39–44 rainbow trout between 100–200 g following the infection method of González et al. (2000), with a larval density of 1500 per tank. Rainbow trout in running seawater were fed daily on a 1% body weight pellet diet. Water temperature was recorded on an hourly basis by means of a Stow Away computerized optical thermometer model WTA08-5 + 37. A variable number (1–16) of fish were sacrificed periodically and the number, developmental stages and body length of juveniles were assessed using a Wild dissecting microscope and graduated eyepiece. Parasites were fixed in a 10% formalin solution in seawater. The development stages were measured using a scaled eyepiece. Measurements are given as mean and range in micrometers, and comprise the body length between the anterior margin without frontal filament and the posterior margin without considering the caudal setae of urosome. A total of 2043 *C. rogercresseyi* at different development stages were collected from a total number of 265 fish. The percentages of the different larval stages were calculated using the total number of parasites collected on the fish samples at each date (n ranged from 2 to 269).

The life cycle duration was measured in days and the incubation temperature (in °C) was measured in the water of the tank or beaker in the case of larvae. The number of days needed to reach the maximum proportion of a stage was registered for copepodids, chalimus 1–4, adult males and adult females. In the case of females with first egg strings and females with pigmented eggs, the time needed for first appearance was registered. These data were used to calculate the rate of development which is the reciprocal of the number of days required for completion of development. The linear regressions of development rate versus the temperature between the range tested, 10–17 °C, were calculated according the Simple Degree–Day Model of Sharov (1998):

$$y = a + bt$$

where y is the development rate and is the reciprocal of the time in days, a is the intercept of the line regression, b is the slope and t is the average temperature of water (in °C).

In this function, when the line intercepts x-axis, t_{min} or the lower temperature limit is reached where the development rate is zero.

Other terms used with the model are:

effective temperature = registered temperature − t_{min} and

S or degree–days (dd) is the effective temperature multiplied by the number of days required to complete development. S does not depend on temperature and is the inverse of the slope: $S = 1/b$.

The cumulative temperature specified as degree–days (dd) was considered from the time of egg hatching. The complete development, cycle or generation time starts from egg hatching and goes until new ovigerous females with mature eggs (pigmented egg) are obtained.

3. Results

3.1. Life cycle of C. rogercresseyi

Fig. 1 shows the life cycle of *C. rogercresseyi*. Eight development stages were found: three planktonic and five parasitic. The planktonic stages comprise two nauplius and a copepodid, the infective stage. The copepodid settles on the host, holding on with its hooked pair of antennae. During moulting, the copepodid extrudes its frontal filament to attach itself permanently to the fish. The parasite moults into the four different chalimus stages always attached by a frontal filament. The size of the parasite increases at each stage. The planktonic stage begins with the first nauplius whose average length was 425 μm (range: 450–375; based on 21 specimens) that moults in the second nauplius with an average length of 463 μm (range: 413–562; based on 24 specimens) and then to the copepodid of 658 μm in average length (range: 600–717; based on 19 specimens). The parasitic stage begins with first chalimus up to fourth chalimus ending in the adult females and males (Fig. 1).

The main characteristics used to identify the first chalimus were the cephalothorax with rounded posterior margins (Fig. 1, ch1) and frontal filament with a rounded base (Fig. 2A) as defined by Kim (1993) and Piasecki and MacKinnon (1993) for other species of *Caligus*. The average length was 830 μm (range: 700–900) based on 21 specimens. The cephalothorax of the second chalimus has posterolateral corners (Fig. 1, ch2). The frontal filament has two bases: a distal one corresponding to the first chalimus and a proximal one, newly formed and bilobed (Fig. 2B). The lobe of chalimus 2 totally covers the lobe of the previous stage. The average length was 1271 (range: 1150–1400) based on 12 specimens. The third chalimus with a marked frontal plate on comparison to the previous stage has a three-based frontal filament and shows sexual dimorphism in the antennae (Fig. 2C). Sex prevalence was recorded only in chalimus 4 because of the difficulty in sex identification in some chalimus 3. In the case of males, the antennae are thick and rounded on the top (Fig. 2D), while in females, they are thinner and end in a claw (Fig. 2E). The fourth appendage are rounded with poorly developed setae. The average length of third chalimus was 2146 μm (range: 1900–2450) based on 33 specimens. The fourth chalimus shows an even greater development of the frontal plate with certain signs of the lunules, which are characteristic of the adult stages (although these may also be absent). The frontal filament of the fourth chalimus presents four bases and the sexual dimorphism of the antenna is apparent (Fig. 2D and E). The genital segment also presents sexual dimorphism. The female has a square genital segment with posterolateral corners and the average length was 4201 μm (range: 3675–4550) based on 18 specimens. The segment in male is rounder and barrel shaped and its average length was 3202 μm (range: 2850–3650). The fourth pair of appendages is divided into two segments and does not show the entire armature as in the adults. Young adults may show remains of attached frontal filament, and the genital segment is more highly developed than in the previous stage but less so than in mature adults. The frontal filament has five bases (Fig. 2F). The proximal base is of transparent material and globe shaped. Many young females carry spermatophores. The genital segment on fertilized females is enlarged due to the production of eggs. However, it is not as easy to differentiate young males from adults as it is in females. The average length of young adult females was

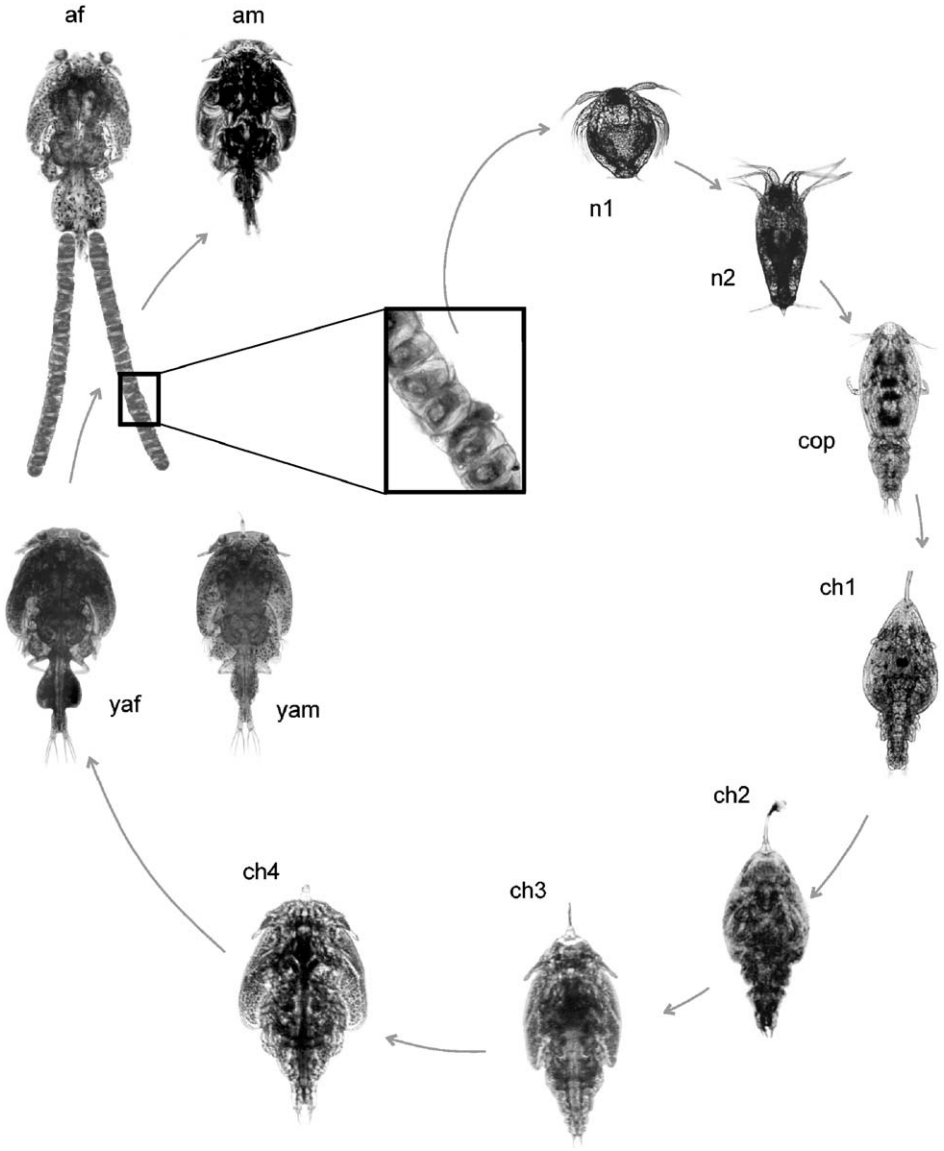


Fig. 1. Life cycle of *C. rogercresseyi*. es: egg strings; n1: first nauplius (0.43 mm long); n2: second nauplius (0.46 mm long); cop: copepodid (0.66 mm long); ch1: first chalimus I (0.83 mm long); ch2: second chalimus (1.27 mm long); ch3: third chalimus (2.15 mm long); ch4: fourth chalimus (3.15 mm long); ya: young adult which is not a different stage from adults (4.1 mm long); am: adult male (4.83 mm long); af: adult female with egg strings (4.79 mm long).

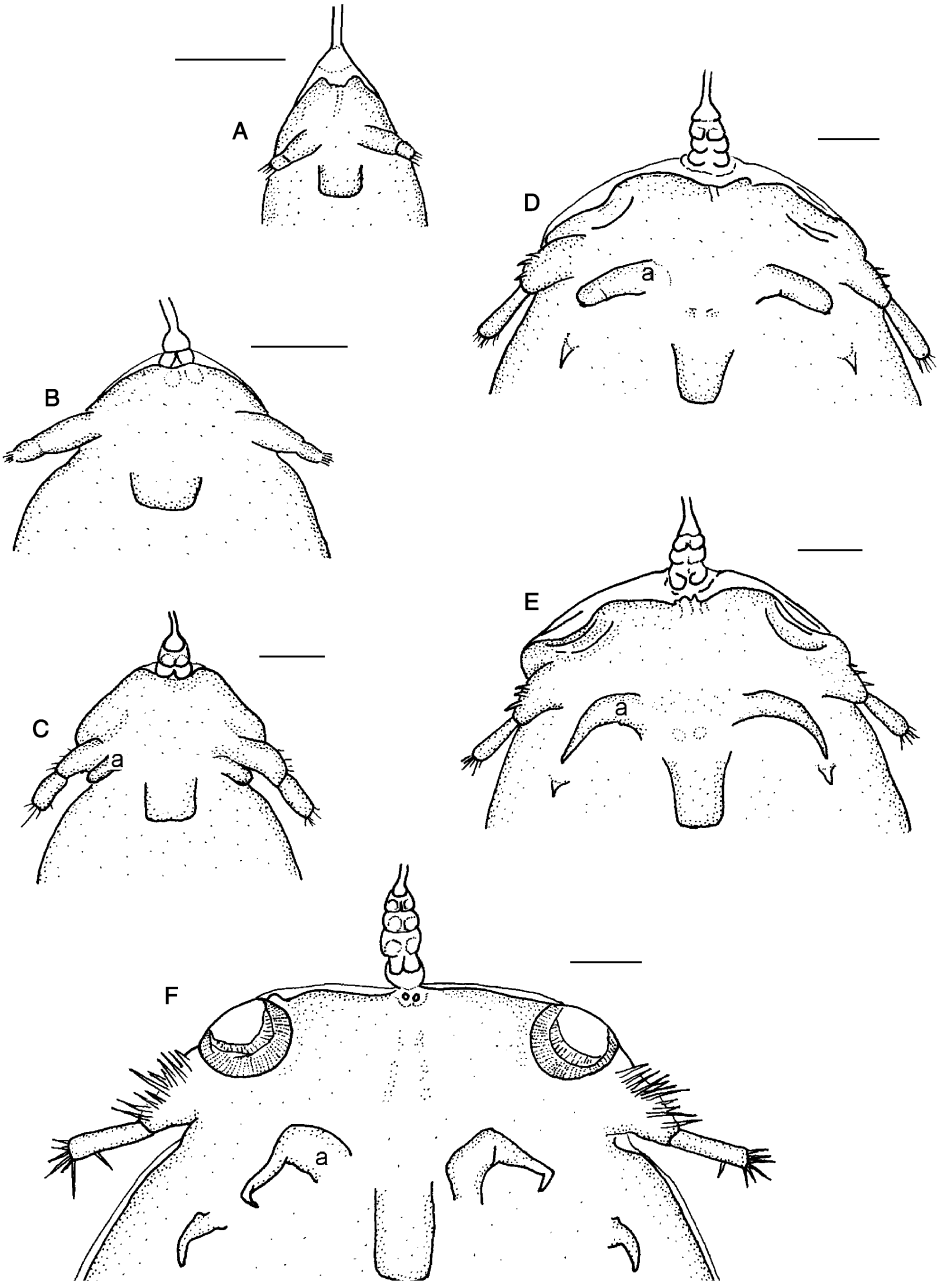


Fig. 2. *C. rogercresseyi*. Ventral and frontal region of cephalothorax showing differences in base of frontal filament and antennae. (A) chalimus 1, (B) chalimus 2, (C) chalimus 3 (a: antennae), (D) chalimus 4 male (a: antennae), (E) chalimus 4 female (a: antennae); (F) adult female (a: antennae). Scale: 200 μm.

4201 μm (range: 3675–4550) based on 18 specimens, while in males, 3956 μm (range: 3250–4600) based on 12 specimens. The cephalothorax presents a transparent cuticle at the margin and fully developed lunules. The fourth pair of appendages is extended and divided into three segments with well-developed setae and spines. Fully mature adults are detached from the host, which allows them mobility over its surface or between different hosts. Adult females defined as "af" in Fig. 3 correspond to young adult females, plus adult females with fully enlarged genital segment, plus females with immature and pigmented eggs. The condition "afe" corresponds to females with egg sacs growing outside the genital complex, but whose eggs are still not viable or are immature. Eggs later mature taking on a pigmented tint when the nauplius is ready to hatch (afpe). In some females considered as "afpe", eggs had already hatched. Average length of reproductive adult females was 4789 μm (range: 4350–5300) based on 47 specimens, and in adult males, it was 4825 μm (range: 4300–5200) based on 26 specimens. It is more difficult to identify mature stages in males since apparently mature individuals could be small as well as large in size. Therefore, the

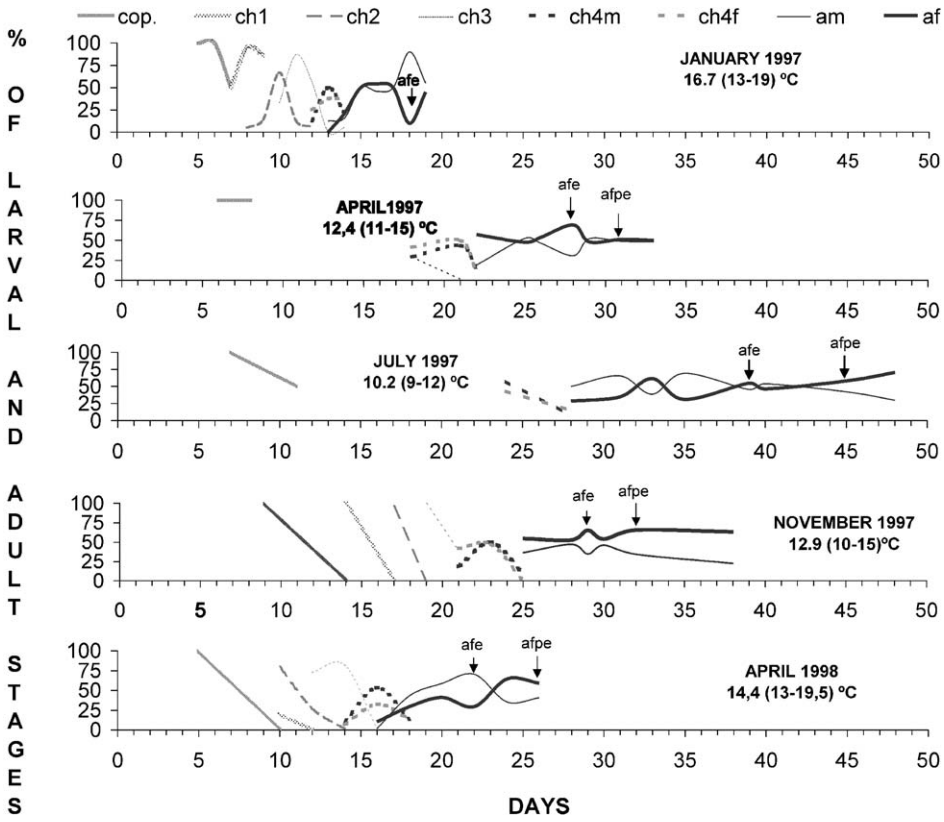


Fig. 3. *C. rogercresseyi*. Frequency of occurrence of different stages in samples. Cycle in days. cop: copepodid; ch: chalimus; ch4m: chalimus 4 male; ch4f: chalimus 4 female; am: adult male; af: adult female; arrow: first appearance of adult females with egg strings (afe) and adult females with pigment eggs (afpe).

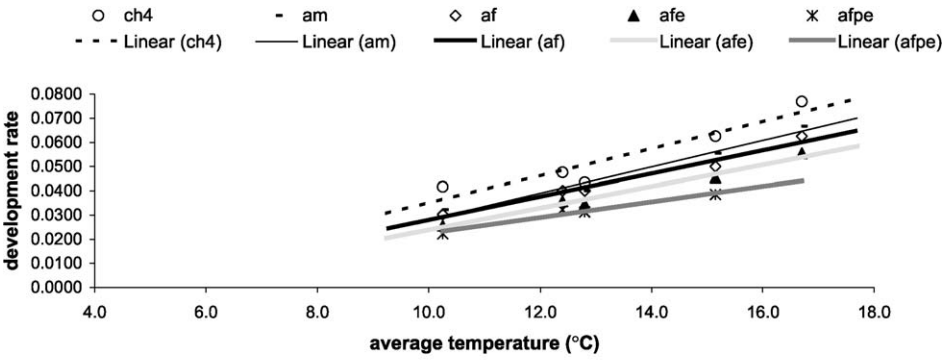
Table 1
Developmental timing in days of *C. rogercresseyi* in the five range of temperature tested. *T* is the time in days required to reach the maximum proportion of chalimus 4, adult male and adult female in Fig. 3 and the time of first appearance of afe (adult females with eggs) and afpe (adult females with pigmented eggs). 1/*T* is the development rate. Temperature 4.2 °C, the critical temperature, was estimated by regression functions in Fig. 4 and Table 2

Average temperature	ch4		am		af		afe		afpe	
	T	1/*T*	*T*	1/*T*	*T*	1/*T*	*T*	1/*T*	*T*	1/*T*
10.3	24	0.0417	31	0.0323	33	0.0303	39	0.0256	45	0.0222
12.4	21	0.0476	25	0.0400	25	0.0400	28	0.0357	31	0.0323
12.8	23	0.0435	25	0.0400	25	0.0400	29	0.0345	32	0.0313
15.2	16	0.0625	18	0.0556	20	0.0500	22	0.0455	26	0.0385
16.7	13	0.0769	15	0.0667	16	0.0625	18	0.0556		
4.2		0		0		0		0		0

complete formation of the genital complex and the presence of inner spermatophores were considered more important.

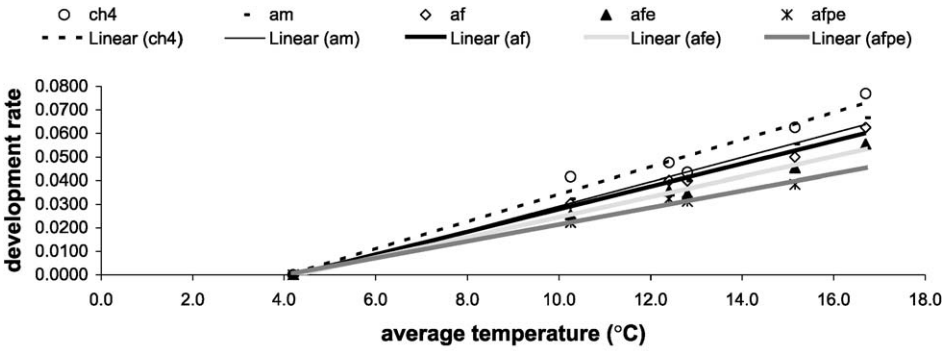
3.2. Generation time

Population curves of the development stages of *C. rogercresseyi* on rainbow trouts were shown in Fig. 3. Population proportions were estimated by observing the proportion of



Developmental Stage	b	a	r^2	p_b	p_a	Minimum Temperature
ch4	0.00562	-0.0212	0.8872	0.01665	0.27149	3.8
am	0.00546	-0.0265	0.9673	0.00254	0.04392	4.9
af	0.00480	-0.0200	0.9725	0.00195	0.05131	4.2
afe	0.00453	-0.0216	0.9808	0.00114	0.02283	4.8
afpe	0.00327	-0.0103	0.9567	0.02187	0.2419	3.2
(median value)						4.2

Fig. 4. Linear regression of development rate (1/*T*) versus temperature (°C) in the five development experiments to estimate lower temperature threshold. Ch4 corresponds to chalimus 4 development, am to adult males, af to adult females, afe to first egg string appearance and afpe to first pigmented egg appearance. In the table, *b* is the slope, *a* the intercept, r^2 the degree of freedom corrected coefficient, *p* the probabilities of each parameter.



Developmental Stage	b	a	r^2	p_b	p_a	Minimum Temperature	Degree-days S= 1/b
ch4	0.00581	-0.0239	0.9493	0.00035	0.02102	4.111	172
am	0.00517	-0.0225	0.9820	0.00004	0.0027	4.352	193
af	0.00481	-0.0202	0.9879	0.00002	0.00146	4.193	208
afe	0.00433	-0.0187	0.9894	0.00002	0.001	4.329	231
afpe	0.00361	-0.0147	0.9835	0.00032	0.00686	4.068	277
(median)						4.2	

Fig. 5. Improved linear regression function of development rate ($1/T$) versus temperature (°C) for chalimus 4 (ch4), adult male (am), adult females (af) first egg appearance (afe) and first pigmented eggs appearance (afpe). The regression was calculated adding to data the estimated critical temperature when development rate is zero. In the table, b is the slope, a the intercept, r^2 the degree of freedom corrected coefficient, p the probabilities of each parameter and S the degree–days required to reach the developmental stage.

each stage present on the fish samples. In an experimentally infected cohort, up to three developmental stages could be present simultaneously. Considering the life cycle in terms of days, the cycle time is shorter in summer (January: more than 18 days) and longer in winter (July: 45 days). The average temperature recorded in the whole cycle during the five experiments (in beakers and in the tanks) were 16.7 °C (range: 13.4–19.3) in January 1997, 12.4 °C (range: 10.8–14.9) in April 1997 and 15.15 °C (range: 12.9–19.5) in April 1998, 10.25 °C (range: 8.7–12.1) in July 1997 and 12.8 °C (range: 7.5–15.1) in November 1997.

The days needed to arrive to a main proportion of chalimus 4 of adult males and adult females and to the first appearance of adult females with egg strings and pigmented eggs

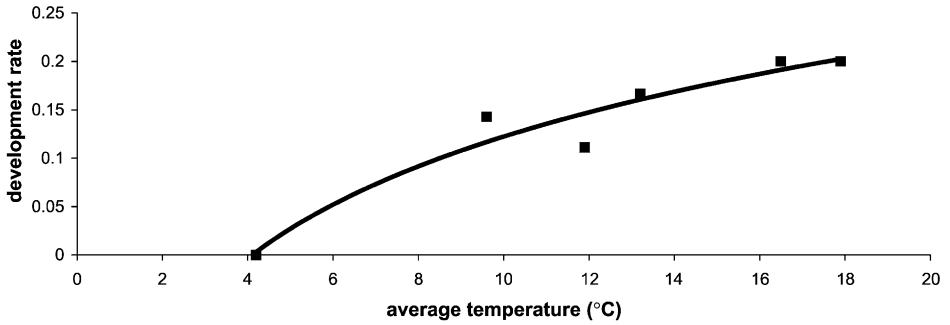
Table 2
Predicted data of developmental time of *C. rogercresseyi* in days, using the simple degree–day models for chalimus 4 (ch4), adult male (am), adult females (af), first egg appearance (afe) and first pigmented eggs appearance (afpe)

Development stage	Degree-days	Predicted development time (days) = S/(temperature − 4.2)					
	S	8	10	12	14	16	18
ch4	172	45.3	29.7	22.1	17.6	14.6	12.5
am	193	51	33.4	24.8	19.7	16.4	14.0
af	208	55	35.9	26.7	21.2	17.6	15.1
afe	231	61	39.9	29.6	23.6	19.6	16.8
afpe	277	73	47.8	35.5	28.3	23.5	20.1

Table 3
Degree–days calculation by accumulating effective temperatures day by day to each development stage in the five experiments. Median and maximum–minimum range of data

Average temperature								
Development stages	10.25	12.4	12.8	15.15	16.7	Median	Maximum	Minimum
ch4	131	174	175	179	167	174	179	131
am	171	209	195	201	188	195	209	171
af	181	209	195	222	199	199	222	181
afe	222	235	233	244	225	233	244	222
afpe	258	258	258	285		258	285	258

were related to the average water temperature. The development rates (the inverse of the number of days, Table 1) were positive and highly correlated to the average temperature (Fig. 4). Using the Simple Degree–Day model, linear functions of development rate versus the average temperature were calculated for chalimus 4, adult male and adult female stages, as well as for the conditions afe and afpe (Fig. 4). No enough data were available to calculate these functions for chalimus 1, 2 and 3. The corrected degree of freedom coefficients of models obtained show a good fit ($r^2 = 0.89$ for chalimus 4; $r^2 = 0.97$ for am; $r^2 = 0.97$ for af; $r^2 = 0.98$ for afe; $r^2 = 0.95$ for afpe). These models allowed to estimate the lower temperature threshold of the caligid development. The median of these minimum temperatures calculated for each function was 4.2 °C. This data was used to fit improved linear regression that gave better corrected degree of freedom coefficients and significant probabilities ($p_b < 0.001$; $p_a < 0.05$) for each parameter of the functions (Fig. 5). The accumulated temperature over the critical minimum, the degree–days calculated from the linear models, were 172 dd to reach chalimus 4 stage, 193 dd to adult male, 208 dd to adult



Model	b	a	r^2	p_b	p_a	Minimum Temperature
y = a+blnx	0.13760	-0.0194	0.8791	0.00202	0.01465	4.2

Fig. 6. Logarithmic model of development rate ($1/T$) versus temperature (°C) for larval stages from hatching to infection of *C. rogercresseyi*. In the table, *b* is the slope, *a* the intercept, r^2 the correlation, *p* the probabilities of each parameter.

female, 231 dd to adult female with first eggs and 277 dd to adult females with pigmented eggs. These data allow to calculate the number of days that takes the total life cycle, as well as the development timing of *C. rogercresseyi* from hatching to the different stages, at any temperature of the water within the range of 4–17 °C tested (Table 2). The degree–days were also calculated in Table 3 by accumulating effective temperatures day by day to each development stage in the five experiments. The median of the value and maximum–minimum range of data were: 174 dd for chalimus 4 (range: 131–179); 195 dd for adult male (range: 171–209); 199 dd for adult females (range: 181–222); 233 dd for (range: 222–244) adult female with first eggs and 258 dd for adult females with pigmented eggs (three experiments, 258, and one, 285).

Analyzing the larval data separated from the parasitic stages, the development is also proportional to the temperature, but not linear (Fig. 6). More days are needed to complete the larval development. The temperature recorded for this period of time is more variable compared to the temperature of whole life cycle, at lower temperature. Copepodids ready for settling were obtained between 5 days at 16.5 °C, 7 days at 9.8 °C to 9 days at 12.1 °C (Table 4). During the planktonic phase at 3 days, a larger portion of larvae are in the nauplius 2 stage and the first copepodids begin to appear. At 5–7 days, almost all larvae have moulted into copepodids. Attachment is better as of day 5 when the accumulated temperature is over the 50 dd of effective temperature. Nevertheless, the settlement and moulting was observed at 9.8 °C when the degree–days are 28. In the other experiments, the larval development ranged from 54 to 62 dd . The presence of an extruded frontal filament without a host has been observed in some copepodids left for a longer period without being infested. At this time, larvae are attached to the glass beaker's wall when the water is changed.

The relation of development rate from hatching to infection versus the average temperature (using the minimum temperature of 4.2 °C) is better represented by a logarithmic curve (Fig. 6). The corrected degree of freedom coefficients of 0.88 shows a good fit and significant probabilities for the parameters of the function ($p_b < 0.002$; $p_a = 0.01$) for each parameter of the functions. Nevertheless, the curve do not allow the

Table 4
Developmental timing of larvae to infection of *C. rogercresseyi* in the five ranges of temperature tested. T is the time in days from hatching to infection. $1/T$ is the development rate. The effective temperature is the accumulated temperature over the minimum 4.2 °C

Date	Temperature		Development to infection	
	Average (°C)	Effective (dd)	T (days)	$1/T$ (days^{-1})
	4.2	0	0	0
July 1997	9.6	27.85	7	0.1429
April 1997	13.2	54.26	6	0.1667
November 1997	11.9	56.4	9	0.1111
April 1998	17.9	68.5	5	0.2000
January 1997	16.5	61.6	5	0.2000
	median	53.7		
	maximum	68.5		
	minimum	27.9		

Table 5
Predicted development time in days of larval stages to infection using the average temperature of water in (°C) and logarithmic model

Temperature (°C)	$y = a + b\ln x$	
	$y = 1/T$ (days^{-1})	T (days)
4.2	0	0
5	0.025	40.0
6	0.05	20.0
7	0.075	13.3
8	0.09286	10.8
9	0.10952	9.1
10	0.12321	8.1
11	0.13571	7.4
12	0.14702	6.8
13	0.15893	6.3
14	0.16964	5.9
15	0.17857	5.6
16	0.18750	5.3
17	0.19524	5.1
17.5	0.20000	5.0

calculation of S as in the linear model. In this case, the number of days was calculated from hatching of larvae to infection, using the average temperature of water and logarithmic model (Table 5) instead of S value.

4. Discussion

The development stages of *C. rogercresseyi* life cycle are described for the first time as well as the most important characters for their identification. The adults were described before by Boxshall and Bravo (2000) for taxonomical identification on the basis of material collected from rainbow trout (*O. mykiss*), Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*) from netpens in Chile. The average length of adult females described by Boxshall and Bravo (2000), 4.96 mm (range: 4.46–5.49 mm), is similar to the length observed in the present work, 4.79 (4.35–5.3) mm. The adult males are slightly longer than adult females.

The present study revealed that *C. rogercresseyi* has eight development stages separated by a moult. No preadult exists. In the family Caligidae, the number of stages may vary between 8 and 11 (Kim, 1993; Lin and Ho, 1993) because preadult stages can be present or not, or because young adult can be mistaken for preadults. The most useful feature to differentiate each development stage was the morphology of the proximal portion of the frontal filament. The number of the lobes produced after each moult indicates the chalimus number. Nevertheless, it must be noted that the paired lobes of the second chalimus totally cover the round lobe of the first chalimus. In the young adult, the four nodules of chalimus 4 were clear, but a fifth pair of nodules can be seen within the extension of the filament base that formed a transparent and globe-like structure. This fifth pair of lobes could

indicate another stage before the adult. On the other hand, when young adult stages are graphed separately in Fig. 3, no clear Gauss curve can be seen to occur with chalimus stages, indicating thus that they are not separated stages. This fifth pair of lobes at the filament end was also observed in *Caligus elongatus* by Piasecki and MacKinnon (1993), whom initially accepted the presence of a chalimus 5. Nevertheless, Piasecki, 1996 only described four chalimus stages and the adult in the development stages of this species and the absence of preadult and a chalimus 5. This fifth pair of lobes would be produced after the moult to adult and be present in young adult with filament. This last fall down later, leaving sometime the swollen marginal cuticle that finally completely detaches.

The present studies revealed that the development of *C. rogercresseyi* is temperature dependent and that Simple Degree–Day model can be used to estimate the life cycle duration at a temperature range of 4–17 °C of the water. Studies in pest management showed that in poikiloterm organisms, the temperature controls the development rate. The phenology models (the models that predict time of events in an organism's development) were firstly developed for plants and invertebrates, including insects and nematodes. It was observed that these organisms require a certain amount of heat to develop from one point in their life cycles to another. This measure of accumulated heat is known as physiological time and does not vary. The combination of temperature and time will always be the same, and often expressed and approximated in units called degree–days. At very high and very low temperature, the development do not occur. These temperatures called upper and lower developmental thresholds have been determined for some organisms through carefully controlled laboratory and field experiments. Thresholds vary with different organisms. (Wilson and Barnett, 1983; UC IPM, 2002).

For development, only the effective temperature is useful, calculated as the average temperature registered minus the minimum temperature. The addition of the effective temperatures day by day during the time required to complete a cycle or a development stage gives the value of degree–days, which are temperature independent (Sharov, 1998). The degree–days allow predicting the development time of the parasite in waters with different temperatures.

At moderate temperatures, as stated by Sharov (1998), the development rate is a linear function of temperature and the Simple Degree–Day Model can be used to estimate the time in one life cycle event. Deviation points occurred at temperatures that are too low or too high. One of the assumption of this model is that the temperature must be constant, to allow the average of temperatures. In the case of more variable temperature, where the average of temperatures cannot be done, more complicated nonlinear models are used.

The minimum temperature at which there is no development and all stages of *C. rogercresseyi* died was calculated as 4.2 °C with a maximum–minimum range of 3.2–4.9 °C. The range of temperature used in the studies, 10–17 °C, did not allow estimating the upper development threshold, at which the development rate slow down or was reduced. Nevertheless, 17 °C is a high value to the average temperature of the sea in the South of Chile. Then, the calculation of the upper temperature limit would not be relevant in this case.

According to this information, the number of days that last a life cycle is not a good basis for making management decisions. Degree–day monitoring provides a physiological time scale that is biologically more accurate (UC IPM, 2002). The data obtained in this

work only allowed the estimation of development over chalimus 3 stages. The timing to chalimus 4 stages, 172 dd, is important because this is the development stage before the parasite begins to reproduce. This lag time should be considered as maximum between chemical treatments to prevent the sea lice infestation and dissemination if the parasite development is synchronic. At 10–14 °C this period should be 30–18 days. The whole life cycle from hatching to the females with pigmented eggs was 277 dd using the model and 258 dd in average (three experiment gave 258 dd, and one, 285 dd) adding the effective temperatures at each experiments. At 10–14 °C of water temperature, the life cycle will take 48 and 28 days, respectively, in the case of 277 dd, and 45 and 26 days, respectively, in the case of 258 dd.

Although Piasecki and MacKinnon (1995) used the degree–days to describe the development of *C. elongatus*, the variable corresponds to the accumulation of total temperature which is temperature dependent. In their experiments, they only tested one temperature, with which they cannot estimate the minimum development temperature and neither calculate the effective temperature. The generation period of 43.3 days at 10 °C or 433 dd estimated for *C. elongatus* under experimental conditions is similar to the one observed in the present study (45 days at 10.2 °C). Hogans and Trudeau (1989) obtained similar results of 5 weeks at 10–12 °C when studying *C. elongatus* in pen-reared salmon in Canada. Tully (1989), however, on studying *C. elongatus* under natural conditions on pen-reared salmon and similar temperature estimated a generation time of 77 days, which is considerably longer. Although the author also calculated the generation time by its relationship with temperature, he worked with days and not with development rates that are linear dependent. In addition, he worked with entire populations of parasites that could phase out in time. The development cycles are not as simultaneous as they almost are in the cohort of the studies under laboratory conditions.

Lepeophtheirus salmonis took 19 days at 17 °C to complete one generation period in Scotland, 43 days at 10 °C and 93 days at 5 °C following the life cycle under experimental conditions in tank-reared salmon and induced infestation (Wadsworth et al., 1998). These results are also similar to those obtained with *C. rogercresseyi* that has 8 developmental stages instead of 10. Temperatures as low as 5 °C occurred in the Northern Hemisphere (Tully, 1992; Wadsworth et al., 1998), but in the Xth Region in Chile, where most fish farms are found, the water temperatures do not fall below 8–9 °C (Phytoplankton Monitoring Program from INTESAL, Alejandro Clément, personal communication). Therefore, 73-day cycles at 8 °C, respectively, should be expected in winter using the degree–day model. Average summer temperatures of 14 °C and somewhat lower temperatures of 12 °C during the rest of the year would provide 28 and 36 days generation periods (including larval development).

The most important feature observed as indicator of development during the incubation of planktonic stages in the laboratory was the moulting of larvae to copepodids, and then, its ability to settle down. Then, comparing the effective temperature accumulated, it was surprising to note the lower temperature accumulated for the winter experiment in relation to the other experiments (28 dd). Piasecki and MacKinnon (1995) registered 38 dd (most probably of total temperature and not effective temperature) from hatch to copepodid and more than 71–180 dd to moult to chalimus 1 when already infected. The total temperature accumulated in our winter experiment was 67 dd. The model that explains the variation of

development rate with temperature in larval stages, although proportional, is not linear like for chalimus and adults, but logarithmic. In addition, although the development of the caligid larvae was represented only as temperature dependent, there is another factor that can be affecting the duration of life cycle. This is the probability of copepodid larvae to find the host or the occurrence of a delay in the infection process. In our experiments, it was possible to postpone the experimental infections for some days in the case of technical problems. This delay can probably also occur in nature. As we could see in the laboratory, copepodids can survive 7 days more at 12.4 °C before dying, if they do not find the host. On the other hand, there was more temperature variation in larval phase compared to the whole cycle that could also be affecting the data.

In the salmon farms, the stages that really damaged farmed fish are copepodids already settled, chalimus and adults. It could be interesting then to evaluate the time of this parasitic period that is subtracted to the whole cycle the larval period. Using the logarithmic model, the development to infection should take 8 and 6 days for 10 and 14 °C, respectively. Then, the development from chalimus 1 to chalimus 4, using the linear model, should take 22 and 12 days at 10 and 14 °C and to females with pigmented eggs, 40 and 22 days, respectively. Using the sum of the effective temperatures, the 258 dd for the whole cycle minus the larval period would take 37 days at 10 °C and 20 days at 14 °C.

Although generation time in caligid copepods is mainly determined by temperature, there are other variables that also affect parasite development such as salinity and host reaction to the parasite. Even though salinity does not in itself retard generation time, it does affect larvae survival, nauplius and copepodids, the more fragile stages of the caligid to low-salinity waters. The geographic areas in Chile with greater maximum and minimum temperature variation, where extreme temperatures of 6.5 °C occur, are located in estuaries where salinity levels are low due to fresh water from rivers. Salinity gradients in these areas can vary from 30‰, 20‰ up to 7‰, and would not allow the survival of the nauplius and copepodid stages which die at salinity levels of 20‰ or lower (González and Carvajal, unpublished data). In these areas, however, adult stages have been observed.

The other factor important in the development of sea lice is the host reaction to the parasite. Experiments on the differential susceptibility of three salmonid species to *C. rogercresseyi* revealed that rainbow trout is the most susceptible species and coho salmon the most resistant. After infecting with the parasite, recent smolts reared in tanks had more adult stages (and less chalimus stages) in rainbow trout than in Atlantic salmon 9 days post-infection. This was also found during farm monitoring (González et al., 2000). Nevertheless, the generation period of *C. rogercresseyi* would be expected to be similar to Atlantic salmon compared to rainbow trout, due to experimental data of *L. salmonis* in Scotland (Wadsworth et al., 1998). In coho salmon, the number of *C. rogercresseyi* adults is scarce although juvenile stages may be found (González et al., 2000). The parasite would not mature on this species, therefore, the appearance of ovigerous females. Horizontal transmission of the parasite can occur between rainbow trout and coho salmon. Proliferation of parasites is, however, expected to be on sick coho salmon, which can harbour an important burden of earlier stages of the parasite. Bruno and Stone (1990) also found that moribund salmon appeared to attract more lice than healthy fish due to the preference of the parasite or selection because they have lowered resistance to sea lice infestation.

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