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The tolerance of *Caligus rogercresseyi* to salinity reduced in southern Chile

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Abstract

A study was carried out to obtain information about the tolerance of *Caligus rogercresseyi* to salinity. Samples of *C. rogercresseyi* were collected from Atlantic salmon (*Salmo salar*) in four areas of southern of Chile (Lat 42°S), which were exposed to different concentrations of salinity. From the results obtained it was evident that *Caligus* displayed sensitivity to salinity lower than 20‰. However differences of tolerance were observed in the parasites collected from Puerto Montt- Castro region compared with parasites collected from Quellón and Hornopiren, on these latter sites freshwater had a major influence and reduced the intensity of infection.

Introduction

Caligus rogercresseyi Boxshall & Bravo is the most important parasite of farmed salmon in the south of Chile since it was recorded for the first time infecting Atlantic salmon (*Salmo salar*) in the spring of 1997. The most susceptible hosts are Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*). However in the last few years' juvenile stages of *Caligus* have also been recorded severely infecting smolts of coho salmon (*Oncorhynchus kisutch*) following transference to seawater. Females of *Caligus rogercresseyi* are smaller (0.5 mm, total length) compared with *Lepeophtheirus salmonis* (12 mm). The life cycle is dependent on temperature and, at 10°C; the development of the parasite is 45 days while at 15°C it is 26 days (Gonzalez & Carvajal, 2003). As *Caligus* displays a lower tolerance to salinity, some farms have been located in estuarine zones to avoid the high load of parasite over the fish.

The economic implications of the severe infections by caligids are mainly associated with the mechanical damage caused by the parasite. Fish show numerous petechial haemorrhaging over the entire body, and these may provide an entry portal for other pathogens. The parasite does not kill the fish, but weakens them, and this leads to diseases such as Infectious Pancreatic Necrosis (IPN) or Salmon Rickettsial Syndrome (SRS). The low condition exhibited by the infested fish is the result of the inappetence shown by the fish because of the high stress imparted by the parasite, and this is also exacerbated by osmotic problems.

A variety of environmental and biological factors, husbandry and managements practices that may influence the abundance and impact of sea lice on farmed salmonids have been identified (Pike & Wadsworth, 1999; Rae, 2002; Johnson et al., 2004). These factors and husbandry practices have been

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used to develop management strategies for sea lice on farmed salmonids (Johnson et al., 2004). Besides temperature, which has been shown to have an important influence in the life cycle of the sea lice, controlling the development times of parasitic copepods (Tully, 1992; Tucker et al., 2000), salinity has also been shown to be important in the abundance of the parasite. Tucker et al. (2000) reported that *Lepeophtheirus salmonis* had a higher growth rate and rate of settlement at a salinity of 34‰ when compared with 24‰. Salinity has also been shown to affect hatching and development of the free-swimming stages of *L. salmonis* (Wootten et al., 1982). Johnson & Albright (1991) found that nauplii were not produced at salinity below 15‰ and that at salinity levels of 25 ‰ and below, less active nauplii were produced with copepodids dying on moult. In British Columbia it was reported that sea lice larvae do not consistently develop to an infectious stage at salinities <30‰ (Brooks, 2005). Pike & Wadsworth (1999) reported that adult lice die rapidly at salinities <12‰ and that, while eggs hatched successfully at salinities as low as 15‰, survival was negligible. Survival improved at 20–25‰, but development to the copepodid stage remained low at salinities <30‰. Heuch et al. (1995) reported that copepodids of *L. salmonis* avoid water with less than 20‰.

There is no documented information about the tolerance of salinity in *Caligus rogercresseyi*, only the records of some farms located in estuarine areas where caligus is not present with salinity lower than 15 ‰ (personal observations). This report gives information about the tolerance of *C. rogercresseyi* to different levels of salinity recorded in the laboratory trial, under controlled conditions.

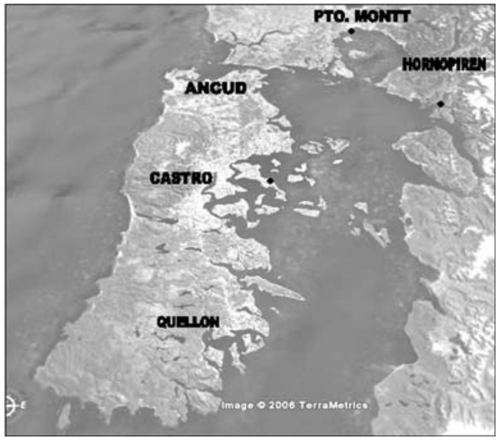

Figure 1. Location of sites from where caligus were collected.

Methodology

Between May and June 2006 samples of adults caligids were taken from four farms located in the south of Chile. One farm was selected from the areas of Puerto Montt, Castro, Quellón and Hornopiren (Figure 1). The first two farms are located in sites with a high level of salinity and the last two in sites with influence of freshwater. Fish were anaesthetized with 15 ppm of benzocaine and parasites were gently removed, stored in a container supplied with aeration and kept at low temperature with ice pack in a box and transported to the laboratory at the Aquaculture Institute of the Universidad Austral de Chile in Puerto Montt, where the salinity challenges were carried out.

At the laboratory, five males and five females were exposed to different gradient of salinity (0; 5; 10; 15; 20 and 31‰) and maintained in containers supplied with constant aeration. Mortality was recorded at 30 min, 1 hour, 12

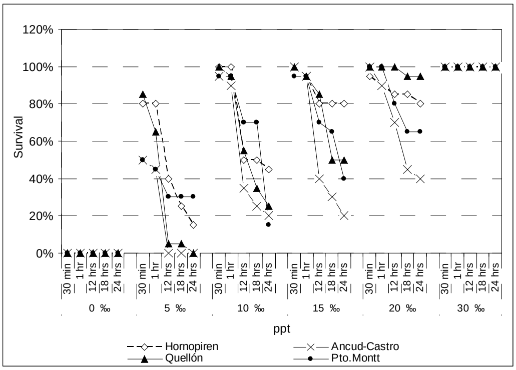

Figure 2. Tolerance of *Caligus rogercresseyi* at different salinity gradients.

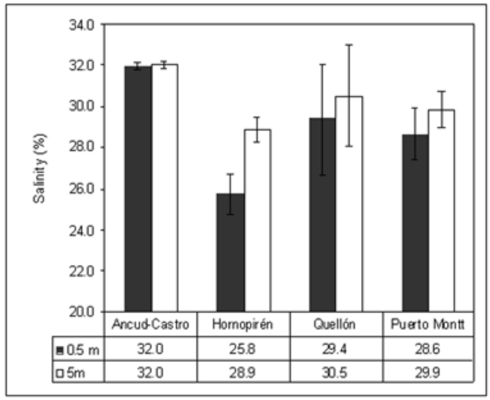

Figure 3. Salinity (‰) recorded in the four study sites at 0.5 m and 5.0 m depth.

hours; 18 hrs and 24 hrs respectively. The mix of salinity gradient was obtained using seawater at 31‰ previously filtered and mixed with fresh water obtained from a river near the sites. Salinity concentrations were measured with a digital salt meter YSI Mod. EC300CC-04. Temperature was also recorded during the period of challenge.

Confidence limits of 95 % were estimated for the parameters. Analysis of variance and the Multiple Range Test, (LSD) Fisher was used for estimating differences between sites.

Results

After 30 minutes of exposure at 0‰, survival was 0% in all the samples of *C. rogercresseyi* obtained from the four areas, while all parasites survived at 31‰ after the 24 hours of exposure (Table 1; Figure 2). Parasites obtained from the area of Ancud-Castro showed lower tolerance to salinity of 5‰ than the parasites collected from the other three areas. At 15‰ the survival of parasite collected from Hornopiren was 80% after 24 h of exposure, while for parasites collected from Ancud-Castro this was 20%.

Hornopiren was the area where *Caligus* showed major tolerance to low salinity (Table 1) and also was the area with the higher fluctuation of salinity during the year, while in comparison the Ancud-Castro area showed less fluctuation at both depths (Figure 3). At 20‰, females showed more tolerance to salinity than males (Figure 5). However, significant differences in salinity tolerance between males and females (Table 3) were not recorded in the different areas ($p<0.33$).

The salinity gradient through the full year showed a similar pattern of distribution at 0.5 and 5.0 m, with significant differences between both depths ($p<0.05$). Hornopiren showed the lower gradient of salinity with respect to the other areas and the lowest prevalence of *C. rogercresseyi* was recorded between June to November and the low intensity of infestation (Figure 6). The higher prevalence was recorded in the areas of Ancud-Castro and Puerto Montt, However the highest rate of infestation was recorded in the area of Quellón (Table 4; Figure 6). All areas showed a decrease of temperature from May to September, a period which comprises

Salinity	Exposure time	Survival			
		Pto.Montt %	Ancud-Castro %	Quellón %	Hornopire %
0 ‰	30 min	0	0	0	0
	1 hr	0	0	0	0
	12 hrs	0	0	0	0
	18 hrs	0	0	0	0
	24 hrs	0	0	0	0
5 ‰	30 min	50	50	85	80
	1 hr	45	45	65	80
	12 hrs	30	0	5	40
	18 hrs	30	0	5	25
	24 hrs	30	0	0	15
10 ‰	30 min	95	95	100	100
	1 hr	95	90	95	100
	12 hrs	70	35	55	50
	18 hrs	70	25	35	50
	24 hrs	15	20	25	45
15 ‰	30 min	95	100	100	100
	1 hrs	95	95	95	95
	12 hrs	70	40	85	80
	18 hrs	65	30	50	80
	24 hrs	40	20	50	80
20 ‰	30 min	100	100	100	95
	1hr	100	90	100	90
	12 hrs	80	70	100	85
	18 hrs	65	45	95	85
	24 hrs	65	40	95	80
30 ‰	30 min	100	100	100	100
	1 hr	100	100	100	100
	12 hrs	100	100	100	100
	18 hrs	100	100	100	100
	24 hrs	100	100	100	100

Table 1. *Caligus rogercresseyi* survival at different salinity gradients in the four areas studied.

the winter season in the southern hemisphere (Figure 4; Table 2). It were not recorded difference in temperature between depths, however, significant differences were recorded between sites (p=<0.03). Hornopiren showed a range of 1.2°C over the temperature recorded in Quellón, for the same year (2006).

Discussion

The differences in the tolerance of sea lice to salinity collected from the four areas in the study seem to be connected with the natural gradient of salinity of each site (Table 1; Figure 3) from which the sea lice were collected. *C. rogercresseyi* collected from Hornopiren

Month	Ancud-Castro				Hornopirén				Quellón				Pto. Montt			
	Temperature		Salinity		Temperature		Salinity		Temperature		Salinity		Temperature		Salinity	
	0.5m	5m	0.5m	5m	0.5m	5m	0.5m	5m	0.5m	5m	0.5m	5m	0.5m	5m	0.5m	5m
Jan.	13,1	13,2	32,1	32,0	15,9	15,5	23,9	28,9	12,6	12,3	32,0	33,0	14,1	14,2	27,2	28,5
Feb.	13,6	13,6	32,2	32,3	15,8	15,1	26,5	29,6	12,6	12,2	32,0	33,0	14,5	14,3	28,3	29,7
Mar.	12,7	12,8	32,0	32,1	14,3	14,2	25,7	29,0	11,8	11,6	32,0	33,0	13,1	13,3	29,0	29,7
April	12,2	12,2	32,1	32,3	12,4	12,3	26,0	28,6	11,6	11,4	29,0	30,5	12,3	12,3	29,8	30,3
May	11,4	11,4	31,9	32,1	10,8	11,2	25,4	28,5	10,9	11,1	30,0	31,0	11,5	11,4	29,7	30,6
June	10,6	10,9	31,9	32,1	9,7	10,1	24,9	28,5	10,5	10,8	26,7	28,0	10,9	11,0	30,2	31,0
July	10,2	10,4	31,9	31,9	9,8	10,0	25,8	28,8	10,5	10,7	24,0	25,5	10,3	10,6	29,2	30,8
Aug.	10,1	10,3	31,5	31,7	9,9	10,3	26,5	29,2	10,2	10,4	26,7	28,0	10,4	10,4	29,5	30,4
Sept.	10,3	10,3	32,0	32,0	10,4	10,5	26,9	29,6	10,1	10,4	28,3	29,5	10,6	10,8	28,6	29,8
Ect.	11,0	11,0	31,7	32,0	11,8	11,6	27,3	29,8	10,9	10,8	28,3	30,0	11,4	11,4	28,8	29,9
Nov.	11,8	11,9	31,8	32,2	13,2	13,5	24,6	27,5	11,0	11,0	31,5	32,0	12,4	12,4	27,7	29,4
Dec.	12,7	12,8	32,2	31,7	14,0	14,1	25,5	28,8	11,6	11,3	32,0	33,0	13,4	13,4	25,8	28,1

Table 2. The monthly average temperature (°C) and salinity (‰) recorded at 0.5 m and 5.0 m depth in the four areas of study.

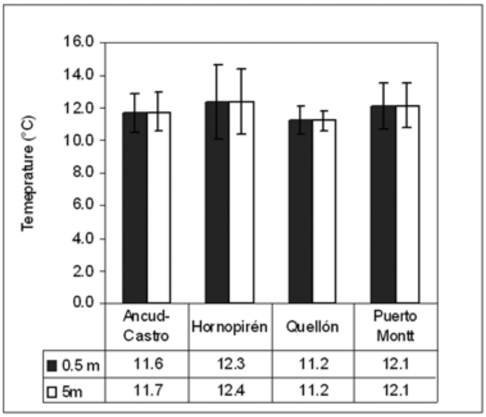

Figure 4. Temperature (°C) recorded in the four study sites at 0.5 m and 5.0 m depth.

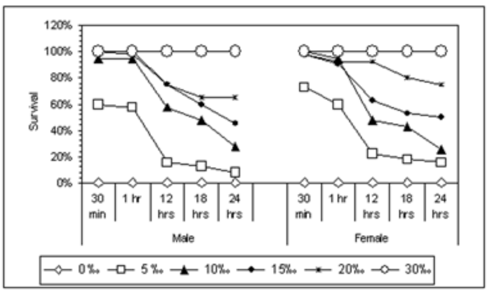

Figure 5. Tolerance of male and female *C. rogercresseyi* , to different salinity gradients.

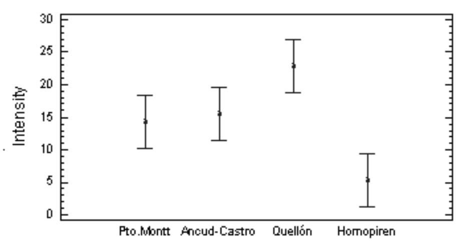

Figure 6. The intensity of infestation of sea lice in the four study sites.

showed the highest tolerance to low salinity, which can be explained because of the high influence of freshwater supplied for several rivers in the zone and the parasites must be adapted to this adverse situation. Hornopiren shows high salinity fluctuations during the whole year (Table 2; Figure 3), with an important difference between the surface and depth because of the freshwater input. Acud-Castro, was the area where the sea lice showed the lowest tolerance to low salinity and the area where less salinity fluctuation was recorded in the whole year and this could explain in some way the higher intensity of infection rates by *Caligus*.

According to the results obtained in this study it can be concluded that besides the seasonal temperature effect, the prevalence and intensity of *C. rogercresseyi* is highly influenced by the salinity gradient, which explains in some way the lower prevalence and intensity recorded in Hornopiren compared with the other three areas of study. According to Tucker et al. (2000), the rate of settlement of copepodids of *L. salmonis* is lower at a salinity under 24‰. In addition, there are antecedents that demonstrate that below 15‰ the hatching and development of nauplii is severely reduced (Wotten et al., 1982; Johnson & Albright, 1991). We observed that *C. rogercresseyi* collected from sites highly influenced by freshwater showed a better adaptation to low salinity in comparison with locations where important fluctuation of salinity through the year were not recorded. Females at 20‰ showed more tolerance to salinity than males (Figure 5; Table 3) which could be explained for the condition of the females to preserve the specie under limited conditions of survival.

It is hoped that this study can contribute to the improvement of the management of sea lice on farmed fish. The information generated from this study should permit a better

Salinity	Exposure time	Survival							
		Pto.Montt %		Ancud-Castro %		Quellón %		Hornopire %	
		Male	Female	Male	Female	Male	Female	Male	Female
0 ‰	30 min	0	0	0	0	0	0	0	0
	1 hr	0	0	0	0	0	0	0	0
	12 hrs	0	0	0	0	0	0	0	0
	18 hrs	0	0	0	0	0	0	0	0
	24 hrs	0	0	0	0	0	0	0	0
5 ‰	30 min	40	60	50	50	80	90	70	90
	1 hr	40	50	50	40	70	60	70	90
	12 hrs	20	40	0	0	0	10	40	40
	18 hrs	20	40	0	0	0	10	30	20
	24 hrs	20	40	0	0	0	0	10	20
10 ‰	30 min	90	100	90	100	100	100	100	100
	1 hr	90	100	90	90	100	90	100	100
	12 hrs	80	60	30	40	70	40	50	50
	18 hrs	80	60	20	30	40	30	50	50
	24 hrs	10	20	20	20	40	10	40	50
15 ‰	30 min	100	90	100	100	100	100	100	100
	1 hrs	100	90	100	90	100	90	100	90
	12 hrs	80	60	50	30	80	90	90	70
	18 hrs	0	50	50	10	20	80	90	70
	24 hrs	40	40	30	10	20	80	90	70
20 ‰	30 min	100	100	100	100	100	100	100	90
	1hr	100	100	90	90	100	100	100	80
	12 hrs	60	100	50	90	100	100	90	80
	18 hrs	60	70	20	70	90	100	90	80
	24 hrs	60	70	20	60	90	100	90	70
30 ‰	30 min	100	100	100	100	100	100	100	100
	1 hr	100	100	100	100	100	100	100	100
	12 hrs	100	100	100	100	100	100	100	100
	18 hrs	100	100	100	100	100	100	100	100
	24 hrs	100	100	100	100	100	100	100	100

Table 3. Survival of *C. rogercresseyi* by sex at different salinity gradients in the four study areas.

understanding of the response of *C. rogercresseyi* to salinity and to make a more accurate prediction of zones of infection and the identification of those farms where the control of sea lice with reduced salinity will be most beneficial.

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	Pto.Montt		Ancud-Castro		Quellón		Hornopirén	
	Prevalence	Intensity Mean (Range)	Prevalence	Intensity Mean (Range)	Prevalence	Intensity Mean (Range)	Prevalence	Intensity Mean (Range)
Jan.	82,7%	8 (0-22)	96,1%	28 (10-68)	82,2%	17 (1-55)	66,7%	18 (12-59)
Feb.	100,0%	46 (7-350)	99,8%	20 (6-46)	86,2%	31 (7-70)	71,6%	8 (1-13)
Mar.	70,0%	5 (0-14)	99,1%	16 (3-45)	99,5%	49 (8-81)	46,1%	3 (0-5)
April	75,0%	7 (0-24)	87,8%	16 (4-39)	84,5%	14 (1-46)	72,5%	6 (2-13)
May	78,0%	5 (0-20)	97,7%	19 (1-56)	99,6%	46 (2-146)	88,3%	8 (2-21)
June	81,7%	10 (2-35)	77,9%	6 (0-19)	82,0%	9 (1-57)	44,0%	3 (0-4)
July	93,4%	14 (1-58)	61,9%	7 (1-15)	67,6%	8 (0-22)	35,0%	3 (0-4)
Aug.	72,6%	6 (1-30)	92,2%	11 (1-34)	77,0%	15 (0-38)	44,0%	3 (0-5)
Sept.	91,3%	15 (1-65)	95,9%	16 (3-37)	78,0%	6 (0-16)	46,7%	4 (1-6)
Oct.	92,7%	17 (2-61)	92,3%	17 (3-49)	64,2%	21 (1-37)	34,0%	3 (0-5)
Nov.	94,8%	14 (3-59)	97,4%	13 (2-38)	70,6%	42 (17-71)	26,7%	3 (0-4)
Dec.	98,3%	25 (4-77)	96,7%	18 (3-44)	98,4%	17 (3-47)	63,0%	4 (2-11)

Table 4. Values of prevalence and intensity of *C. rogercresseyi* infection on salmonids in the four sites under study.

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