



# Evaluation of survival and metamorphosis of larvae of *Caligus rogercresseyi* (Boxshall and Bravo, 2000) (Crustacea, Copepoda) in Chile, depending on temperature, salinity and oxygen

A. Mardones<sup>a,b\*</sup>, V. Gajardo<sup>a</sup>, M. I. Pizarro<sup>a</sup>, A. Augsburger<sup>c</sup>, R. Vega<sup>a,b</sup>, F. Encina<sup>d,e</sup>,  
C. Pichara<sup>a</sup> and P. De los Ríos Escalante<sup>d,e</sup>

<sup>a</sup>Departamento de Ciencias Agropecuarias y Acuícolas, Facultad de Recursos Naturales, Universidad Católica de Temuco, Casilla 15-D, Temuco, Chile.

<sup>b</sup>Núcleo de Investigación en Producción Alimentaria, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Chile.

<sup>c</sup>Universidad Santo Tomás, Sede Puerto Montt, Chile.

<sup>d</sup>Departamento de Ciencias Biológicas y Químicas, Facultad de Recursos Naturales, Universidad Católica de Temuco, Casilla 15-D, Temuco, Chile.

<sup>e</sup>Núcleo de Estudios Ambientales, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Casilla 15-D, Temuco, Chile.

\*e-mail: mardolaz@uct.cl

Received: December 7, 2016 – Accepted: September 21, 2017 – Distributed: May 31, 2019  
(With 6 figures)

## Abstract

The present study assessed the percentage of survival and metamorphosis of larval stages of *Caligus rogercresseyi* (Boxshall and Bravo, 2000) nauplius I, nauplius II to and nauplius II to copepodite, conducting bioassays in triplicate with 50 larvae each, nauplius I or nauplius II, at temperature of 12 °C, 15 °C and 18 °C; salinity 20, 23, 25, 2729, 31, 33 and 35 g/L and oxygen saturation with ranges between 30-60%, 90-100% and 190 - 200%. Bioassays were performed in station Quillaípe of Fundación Chile, Puerto Montt, Chile. It is concluded that the temperature is inversely proportional to the time of metamorphosis and survival of the larvae of *Caligus rogercresseyi*. In salinity is observed that increased this, greater is the percentage of survival and metamorphosis is faster, while the larvae do not survive less than 20g/L. Oxygen saturation ranges indicate that the larvae do not survive at saturations between 30-60%, and it had no differences between 90-100% saturation and 190-200%.

**Keywords:** *Caligus rogercresseyi*, temperature, salinity, oxygen saturation.

## Avaliação de sobrevivência e metamorfose de larvas de *Caligus rogercresseyi* (Boxshall and Bravo, 2000) (Crustacea, Copepoda) no Chile, dependendo da temperatura, salinidade e oxigênio

## Resumo

O presente estudo avaliou a percentagem de sobrevivência e metamorfose dos estádios larvais de *Caligus rogercresseyi* (Boxshall and Bravo, 2000) nauplio I, nauplio II e nauplio II a copepodita, realizando bioensaios em triplicado com 50 larvas cada, nauplio I ou nauplio II. À temperatura de 12 °C, 15 °C e 18 °C; Salinidade 20, 23, 25, 2729, 31, 33 e 35 g / L e saturação de oxigênio com variações entre 30-60%, 90-100% e 190-200%. Os bioensaios foram realizados na estação Quillaípe da Fundação Chile, Puerto Montt, Chile. Conclui - se que a temperatura é inversamente proporcional ao tempo de metamorfose e sobrevivência das larvas de *Caligus rogercresseyi*. Na salinidade observa-se que aumentou esta, maior é a percentagem de sobrevivência e metamorfose é mais rápida, enquanto as larvas não sobrevivem menos de 20g / L. Os intervalos de saturação de oxigênio indicam que as larvas não sobrevivem a saturações entre 30-60% e não têm diferenças entre 90-100% de saturação e 190-200%.

**Palavras-chave:** *Caligus rogercresseyi*, temperatura, salinidade, saturação de oxigênio.

## 1. Introduction

Introduction farms, by being present in the marine environment are affected by ectoparasites on the Chilean native fauna, mainly *Caligus rogercresseyi* (Boxshall and

Bravo, 2000; González et al., 2016). The life cycle of the parasite consists of three planktonic states, nauplius I, nauplius II, infective copepodite, and five parasitic stages,

chalmus I-IV or juveniles and an adult (González and Carvajal, 2003).

Infective states feed on mucus, skin and blood of its host, causing focal and hemorrhagic ulcerative skin lesions, which can be of variable severity and injury of 2 to 5 millimeters in diameter to large ulcers on the body surface, also has the potential to affect growth, fertility, and the survival of their hosts (Boxshall and Bravo 2000; González et al., 2016). In addition to being a vector of bacterial and viral diseases, such ISA, IPN, SRS (Johnson et al., 2004; Oelckers et al., 2014).

According to information provided by Technological Institute of Salmon of Chile (INTESAL), carried out between the years 2000 to 2006, in the Lakes region (39-43° S), there was a decrease in the level of oxygen and an increase in mild in the salinity of the water, being these fundamental factors in the cycle of the parasite, in such a way that this situation in conjunction with other factors (wind speed, water depth, tides, circulation of water, currents, temperature and salinity patterns), productive practices and management could influence the increase of the levels of infestation by *C. rogercresseyi* in salmonid fish culture (Costello, 1993; Rozas & Asencio, 2007). The aim of this work was to evaluate the effect of temperature, salinity, and oxygen saturation, on survival, and metamorphosis of larvae under controlled conditions *Caligus rogercresseyi*.

## 2. Materials and Methods

Adults fish individuals of *Salmo salar* L., were infested by *C. rogercresseyi*, female's ovigerous, which were extracted manually using fine tweezers from previously anesthetized fish with "AQUÍ-S" (proportion 1:1000, active ingredient isoeugenol 50%). To provide an optimal environment, water filtered and sterilized sea was used by a UV intensity of 23800-25000uW/cm<sup>2</sup> with salinity of 31g/L, ambient temperature and constant aeration.

Specimens of *C. rogercresseyi* ovigerous females, were reared in 1 L beakers filled with seawater (31 g/L), each provided on average 50 larvae, the water was changed daily until 2100 larvae each bioassay to triplicate with a starting amount of 50 larvae. Every 3 hours the metamorphosis of nauplius I to nauplius II and nauplius II to copepod nauplius in all bioassays were counted until all metamorphosed or died. Counts and observations for a magnifier with 10x magnification was used, the larvae were collected with a sieve of 77 microns deposited in Petri dishes with 10 ml of water. The temperature was measured every 10 minutes using a Data - Logger, obtaining data with HOB0 program.

Bioassay 1: Effect of temperature: three bioassays were conducted in triplicate at a constant temperature of 12 °C, 15 °C and 18 °C, precipitates prepared in 90 ml of water, with 31g/L salinity and aeration vessels constant. Kept in incubator refrigerator Velp model FTC 90E to maintain invariable temperature.

Bioassay 2: Effect of salinity: there were eight experiences at different salinities (20, 23, 25, 27, 29,

31, 33 and 35 g/L-1), vessels precipitated with 90 ml of water with aeration and constant temperature (15 °C). The vessels were stacked in an incubator Velp model FTC 90E, to maintain stable temperature. To obtain the expected salinity mixed freshwater with seawater or sea salt was added to obtain the desired salinity more accurately it was registered with the oxygen meter, salinity and temperature YSI model 85 - 10 FT.

Bioassay 3: Effect of oxygen saturation: to determine the effect of oxygen saturation plastic trays with 15 L of water is used in a 40 ml container, with a salinity of 33 ppt. Each tray had oxygen and independent sensor diffuser to maintain saturation within the ranks of 30-60%, 90-100% and 190 – 200%.

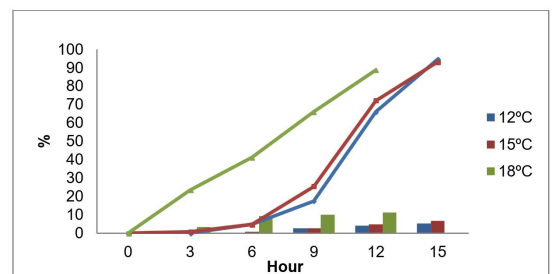
The analysis of the results included first a previous homocedasticity condition using Bartlett test, as previous condition to ANOVA, if it denotes significant differences it was applied Tukey test with a 95% confidence level. Determining if the difference between the evaluated groups is or not significant; P values less than 0.05 indicate significant differences. The SPSS15 program was used.

## 3. Results

### 3.1. Bioassay 1: effect of temperature

Nauplius I at Nauplius II: at 18 °C they metamorphose took 12 hours at temperatures of 12 °C and 15 °C, it took 15 hours. Figure 1 shows that the highest percentage 12 °C metamorphosis, with 95% survival, followed by 15 °C (93%) and 18 °C (89%), the latter having not statistically significantly different temperatures 12 °C and 15 °C according to Table 1.

Nauplius II at Copepod: at 18 °C showed the shortest time of metamorphosis, 36 hours, 15°C followed by 42 hours and finally 12 °C taking 57 hours. According to



**Figure 1.** Metamorphosis nauplius I to nauplius II (lines) and mortality (bars) in percentage, at different temperatures.

**Table 1.** Statistical analysis of the survival in the metamorphosis of nauplii I, nauplii II, at different temperatures.

Temperature (°C)	Temperature (°C)	P
12	15	0.60 n.s
12	18	0.01*
15	18	0.03*

\*The mean difference is significant at 0.05.

Figure 2, at 12°C 91% of the nauplii II they reach their copepodite stage, showing a significant difference with 12 °C and 18 °C, see Table 2.

3.2. Bioassay 2: effect of salinity

Nauplii I to Nauplii II: metamorphosis takes 12 hours to 33g/L and 35g/L, 15 hour between 20g/L, 23g/L, 27g/L, 29g/L y 31g/L and 16 hour to 25g/L time being longer metamorphosis (Figure 3). Nauplii I to II, 99% survival to 35g/L was obtained, showing a significant difference to 20g/L salinity where mortality (53%) exceeds metamorphosis (47%), compared to other salinities (Table 3).

Nauplius II to Copepodite: the minimum time for copepodite was 42 hours at 3 g/L, 45 hours at 20, 31 and 35 g/L, 48 hours at 29g/L, 51 hours at 25 and 25 g/L and it late 54 hours at 23 g/L, being it the maximum time for reach metamorphosis between salinities (Figure 4). The maximum survival was 33 g/L, with 88% and the following was 87% at 35 g/L, and the lower value was 20 g/L with 47%, existing significant differences at salinities analyzed (Table 4).

3.3. Bioassay 3: oxygen saturation effect

Nauplius I to Nauplius II: the metamorphosis late 12 hours at 90-100% and 190-200%, whereas to 30-60% it was not evidenced metamorphosed larvae, and at 9 hours it was observed total mortality (Figure 5). The survival reaches 94% with 90-100% oxygen saturation, with 93% with 190-200%, and it was obtained significant differences between 30-60% (Table 5) due that in this value was observed 100% mortality.

Nauplius II to Copepodite: the metamorphosis late 42 hours with 90-100% and 190-200% oxygen saturation with 30-60% survive 24 hours. The survival reached

87% between the range of 90-100% and 83% between 190-200% oxygen saturation, in the range between 30-60% only reached 3% survival, and this group was significant different (Figure 6, Table 6).

4. Discussion

The results revealed that for metamorphosis in both studied stages, and the survival is temperature dependent, and at high temperature, the metamorphosis time and survival decreased. These results agree with Tully and Whelan (1993), who mentioned that sea temperature increase is directly associated with short time parasite life cycle that in according to Bravo et al. (2009), the presence of low bodies females with low eggs chain. In according to Wootten et al. (1982), Johnson and Albright (1991), Tully and Whelan (1993), Boxaspen (1997), Wadsworth (1998), Pike and Wadsworth (1999), Bravo et al. (2013, 2014) the growth rates, eggs production, survival and recruitment are directly associated to water temperature. Also, the life cycle development was affected by salinity and oxygen saturation.

The salinity effect accelerated the parasite life cycle, at high concentration, the survival increase, at 20 g/L the survival was low. Tucker et al. (2000) described that *L. salmonis* has high growth rate and recruitment at 34 g/L in comparison to 24 g/L salinity. The salinity does not retard the *Caligus* egg time production, but affect the larvae survival in analyzed studies, in according to Gonzalez and Carvajal (2003), salinities lower than 20 g/L does

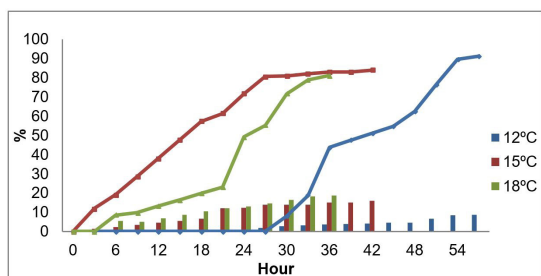


Figure 2. Metamorphosis nauplii II to copepodite (lines) and mortality (bars), as a percentage, at different temperatures.

Table 2. Statistical analysis of the survival in the metamorphosis of nauplii II to copepodite, at different temperatures.

Temperature (°C)	Temperature (°C)	P
12	15	0.07 n.s
12	18	0.02*
15	18	0.59 n.s

\*The mean difference is significant at 0.05.

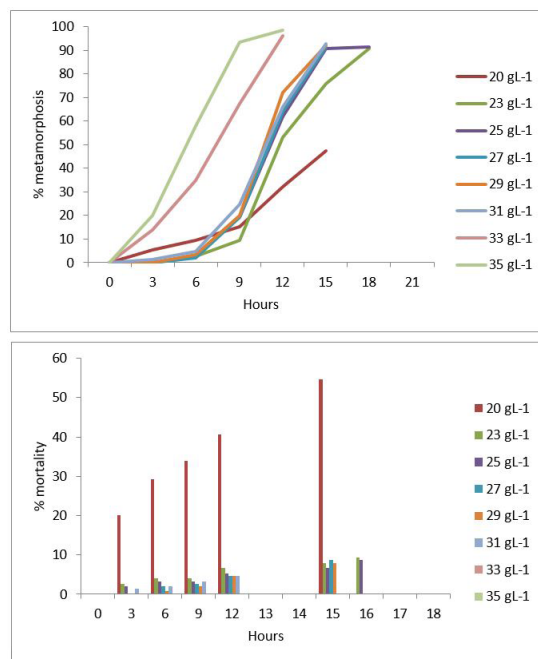


Figure 3. Metamorphosis nauplii I to nauplii II (lines) and mortality (bars), as a percentage, in terms of salinity.

**Table 3.** Statistical analysis of survival in the metamorphosis of nauplii I to II, at different salinities.

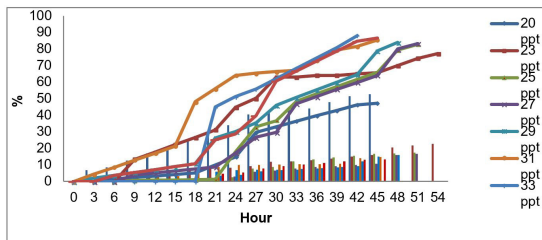
I (Salinity) g/L	J (Salinity) g/L	P	I (Salinity) g/L	J (Salinity) g/L	P
20	23	0.00*	25	27	1.00 n.s
20	25	0.00*	25	29	1.00 n.s
20	27	0.00*	25	31	1.00 n.s
20	29	0.00*	25	33	0.53 n.s
20	31	0.00*	25	35	0.10 n.s
20	33	0.00*	27	29	1.00 n.s
20	35	0.00*	27	31	0.99 n.s
23	25	1.00 n.s	27	33	0.38 n.s
23	27	1.00 n.s	27	35	0.06 n.s
23	29	1.00 n.s	29	31	1.00 n.s
23	31	0.99 n.s	29	33	0.67 n.s
23	33	0.38 n.s	29	35	0.16 n.s
23	35	0.06 n.s	31	33	0.85 n.s
			31	35	0.25 n.s
			33	35	0.94 n.s

\*The mean difference is significant at 0.05.

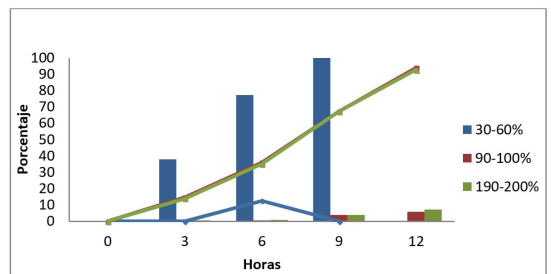
**Table 4.** Statistical analysis of survival in the metamorphosis of nauplii II to copepodite, in terms of salinity.

I (Salinity) g/L	J (Salinity) g/L	P	I (Salinity) g/L	J (Salinity) g/L	P
20	23	0.00*	25	27	1.00 n.s
20	25	0.00*	25	29	1.00 n.s
20	27	0.00*	25	31	1.00 n.s
20	29	0.00*	25	33	0.94 n.s
20	31	0.00*	25	35	0.99 n.s
20	33	0.00*	27	29	1.00 n.s
20	35	0.00*	27	31	1.00 n.s
23	25	0.94 n.s	27	33	0.97 n.s
23	27	0.89 n.s	27	35	0.99 n.s
23	29	0.83 n.s	29	31	1.00 n.s
23	31	0.68 n.s	29	33	0.99 n.s
23	33	0.35 n.s	29	35	1.00 n.s
23	35	0.51 n.s	31	33	1.00 n.s
			31	35	1.00 n.s
			33	35	1.00 n.s

\*The mean difference is significant at 0.05.



**Figure 4.** Metamorphosis nauplii II to copepodite (lines) and mortality (bars), as a percentage, different salinities.

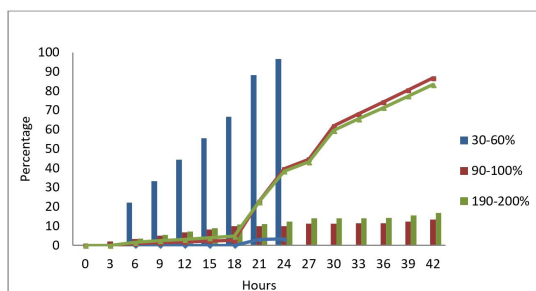


**Figure 5.** Metamorphosis nauplii I to nauplii II (lines) and mortality (bars), as a percentage, different percentages of oxygen saturation.

**Table 5.** Statistical analysis of survival in the metamorphosis of nauplii I to nauplii II, at different oxygen saturations.

(I) Oxygen %	(J) Oxygen %	Sig.
30-60	90-100	0.00
30-60	190-200	0.00
90-100	190-200	0.73

The mean difference is significant at 0.05.

**Figure 6.** Metamorphosis nauplii II to copepodite (lines) and mortality (bars), as a percentage, different percentages of oxygen saturation.**Table 6.** Statistical analysis of survival in the metamorphosis of nauplii II to copepodite, at different levels of oxygen saturation.

(I) Oxygen %	(J) Oxygen %	Sig.
30-60	90-100	0.00
30-60	190-200	0.00
90-100	190-200	0.54

The mean difference is significant at 0.05.

not allow the survival of Nauplius and copepodite stages. It was showed that *C. rogerresseyi* does not survive at low oxygen concentration, and it reach 100% mortality between 30-60% in Nauplius II, and 97% in copepodite stages. At oxygen saturation upper than 90% it was not differences in time and survival. In according to the data base of Phytoplankton Monitoring Program of INTESAL done between 2000 to 2006, in Lakes region it was observed an oxygen decrease and a weak salinity increase, that are main factors in parasite life cycle, and in this scenario, these factors would affect the infestation rate by *C. rogerresseyi* (Rozas and Asencio, 2007; Bravo et al., 2014).

## Acknowledgements

The present study was funded by Fundación Chile the area Recursos Marinos, Aquadvice and project MECESUP UCT 0804, and the authors express their gratitude to M.I for her valuable comments for improve the manuscript.

## References

BOXASPEN, K., 1997. Geographical and temporal variation in abundance of salmon lice (*Lepeophtheirus salmonis*) on salmon (*Salmo salar* L.). *ICES Journal of Marine Science*, vol. 54, no. 6, pp. 1144-1147. [http://dx.doi.org/10.1016/S1054-3139\(97\)80020-3](http://dx.doi.org/10.1016/S1054-3139(97)80020-3).

BOXSHALL, G.A. and BRAVO, S., 2000. On the identity of the common Caligus (Copepoda: Siphonostomatoida: Caligidae) from salmonid net pen systems in southern Chile. *Contributions to Zoology (Amsterdam, Netherlands)*, vol. 69, pp. 137-146.

BRAVO, S., ERRANZ, F. and LAGOS, C., 2009. A comparison of sea lice, *Caligus rogerresseyi*, fecundity in four areas in southern Chile. *Journal of Fish Diseases*, vol. 32, no. 1, pp. 107-113. <http://dx.doi.org/10.1111/j.1365-2761.2008.01012.x>. PMID:19245635.

BRAVO, S., POZO, V., SILVA, M.T. and ABARCA, D., 2013. Comparison of the fecundity rate of *Caligus rogerresseyi* infesting Atlantic salmon (*Salmo salar* L.) on farms in two regions of Chile. *Aquaculture (Amsterdam, Netherlands)*, vol. 404/405, pp. 55-58. <http://dx.doi.org/10.1016/j.aquaculture.2013.04.002>.

BRAVO, S., SILVA, M.T. and TREASURER, J., 2014. Factors affecting the abundance of *Caligus rogerresseyi* (Boxshall and Bravo) on farmed salmonids in Chile in the period 2006–2007. *Aquaculture (Amsterdam, Netherlands)*, vol. 434, pp. 456-461. <http://dx.doi.org/10.1016/j.aquaculture.2014.09.009>.

COSTELLO, M.J. 1993. Review of methods to control sea lice (Caligidae Crustacea) infestations on salmon (*Salmo salar*) farms. In: G.A. Boxshall and D. Defaye. *Pathogens of wild and farmed fish: sea lice*. Chichester: Ellis Horwood, pp. 219-252.

GONZÁLEZ, L. and CARVAJAL, J., 2003. Life cycle of *Caligus rogerresseyi*, (Copepoda:Caligidae) parasite of Chilean reared salmonids. *Aquaculture (Amsterdam, Netherlands)*, vol. 220, no. 1-4, pp. 101-117. [http://dx.doi.org/10.1016/S0044-8486\(02\)00512-4](http://dx.doi.org/10.1016/S0044-8486(02)00512-4).

GONZALEZ, M.P., MUÑOZ, J.L.P., VALERIO, V. and VARGAS-CHACOFF, L., 2016. Effects of the ectoparasite *Caligus rogerresseyi* on *Salmo salar* blood parameters under farm conditions. *Aquaculture (Amsterdam, Netherlands)*, vol. 457, pp. 29-34. <http://dx.doi.org/10.1016/j.aquaculture.2016.01.027>.

JOHNSON, S.C. and ALBRIGHT, L.J., 1991. Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. *Journal of the Marine Biological Association of the United Kingdom*, vol. 71, no. 02, pp. 425-436. <http://dx.doi.org/10.1017/S0025315400051687>.

JOHNSON, S.C., TREASURER, J.W., BRAVO, S., NAGASAWA, K. and KABATA, Z., 2004. A review of the impacts of parasitic copepods on marine aquaculture. *Zoological Studies (Taipei, Taiwan)*, vol. 43, pp. 229-243.

OELCKERS, K., VIKE, S., DUESUND, H., GONZALEZ, J., WADSWORTH, S. and NYLUND, A., 2014. *Caligus rogerresseyi* as a potential vector for transmission of infectious salmon anemia (ISA) virus in Chile. *Aquaculture (Amsterdam, Netherlands)*, vol. 420/421, pp. 126-132. <http://dx.doi.org/10.1016/j.aquaculture.2013.10.016>.

PIKE, A.W. and WADSWORTH, S.L., 1999. Sealice on salmonids: their biology and control. *Advances in Parasitology*, vol. 44, pp. 233-337. [http://dx.doi.org/10.1016/S0065-308X\(08\)60233-X](http://dx.doi.org/10.1016/S0065-308X(08)60233-X). PMID:10563397.

ROZAS, M. and ASENCIO, G., 2007. Assessment of epidemiologic situation of caligiasis in Chile: toward to effective control strategy. *Salmo ciencia. Instituto Tecnológico del Salmón*, vol. 2, pp. 43-59.

TUCKER, C.S., SOMMERVILLE, C. and WOOTTEN, R., 2000. The effect of temperature and salinity on the settlement and survival of copepodids of *Lepeophtheirus salmonis* (Krøyer, 1837) on Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases*,

vol. 23, no. 5, pp. 309-320. <http://dx.doi.org/10.1046/j.1365-2761.2000.00219.x>.

TULLY, O. and WHELAN, K.F., 1993. Production of nauplii of *Lepeophtheirus salmonis* (Krøyer) (Copepoda: Caligidae) from farmed and wild salmon and its relation to the infestation of wild sea trout (*Salmo trutta* L.) off the west coast of Ireland in 1991. *Fisheries Research*, vol. 17, no. 1-2, pp. 187-200. [http://dx.doi.org/10.1016/0165-7836\(93\)90018-3](http://dx.doi.org/10.1016/0165-7836(93)90018-3).

WADSWORTH, S. 1998. *Studies on the biology and control of sea lice Lepeophtheirus salmonis*. Aberdeen, Scotland United Kingdom: University of Aberdeen. PhD Thesis.

WOOTEN, R., SMITH, J.W. and NEEDHAM, E.A. 1982. Aspects of the biology of the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. *Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences*, vol. 81, pp. 185-197. <https://doi.org/10.1017/S0269727000003389>.