Aquaculture 486 (2018) 68–74

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

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

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ARTICLE INFO

Keywords:
Sea lice
Salmon farming
Larval development
Environmental factors

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).

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https://doi.org/10.1016/j.aquaculture.2017.12.017
Received 26 July 2017; Received in revised form 7 December 2017; Accepted 10 December 2017
Available online 12 December 2017

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.