ARTIFICIAL SETTLEMENT OF SEA LICE, *CALIGUS ROGERCRESSEYI* BOXSHALL & BRAVO, 2000 (COPEPODA, CALIGIDAE), ON TISSUES OF FISH USED AS SUBSTRATE

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ABSTRACT

Caligus rogercresseyi is the main ectoparasitic copepod affecting salmon and trout farming in Chile. The aim of this study was to evaluate under laboratory conditions the ability of copepodids of *C. rogercresseyi* to settle on fin, scale, and skin tissues taken from a wild host, the rock cod (*Eleginops maclovinus*) and an exotic host (*Oncorhynchus mykiss*), and evaluate the effects of inductors, such as conditioned water and fish mucus, on the process. The assessment was carried out simultaneously for each host and inductor, using a single pool of larvae (N = 1800). Three replicates per treatment were done and the settlement as well as the development stage of the frontal filament were recorded after 24 h. A total of 341 copepodids were able to settle on the different tissues and hosts. The host showing the higher settlement was *E. maclovinus* (N = 215; 23.9%) compared to *O. mykiss* (N = 126; 14%); the tissue showing the higher number of settled copepodids was the skin (N = 126; 48.4%), followed by fin (N = 140; 41.1%) and scales (N = 36; 10.5%). Regarding inductors, neither conditioned water (N = 112; 32.8%) nor mucus (N = 113; 33.1%) showed significant differences with the control without inductor (N = 116; 34%). Inductors did not have a significant effect on the development of the frontal filament, neither for *E. maclovinus*, nor for *O. mykiss* tissues, thus suggesting the tissues used were able to induce a positive response on these processes. This is the first work reporting a successful settlement of a species of sea louse in tissue extracted from fish.

RESUMEN

Caligus rogercresseyi es el principal copépodo ectoparásito que afecta al cultivo de salmones y truchas en Chile. El objetivo de este trabajo fue evaluar en condiciones de laboratorio la capacidad de los copepoditos de *C. rogercresseyi* de asentarse sobre tejidos de aletas, escamas y piel obtenidos desde un hospedador silvestre (*Eleginops maclovinus*) y desde un hospedador exótico (*Oncorhynchus mykiss*), y evaluar el efecto de inductores tales como el agua acondicionada y el mucus de peces sobre este proceso. La evaluación se realizó para cada hospedero e inductor de forma simultánea, usando un único pool de larvas (N = 1.800). Se realizaron tres replicas por

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tratamiento, registrando el asentamiento y el estado de desarrollo del filamento frontal a las 24 h. Un total de 341 copepoditos lograron asentarse en los distintos tejidos y hospedadores. El hospedador que registró un mayor asentamiento fue *E. maclovinus* (N = 215; 23,9%) en comparación con *O. mykiss* (N = 126; 14%), mientras que el tejido con mayor número de copepoditos asentados fue la piel (N = 165; 48,4%), seguido por el sustrato aleta (N = 140; 41,1%) y finalmente las escamas (N = 36; 10,5%). A nivel de inductores, ni el agua acondicionada (N = 112; 32,8%), ni el mucus (N = 113; 33,1%) presentaron diferencias significativas con el control sin inductor (N = 116; 34%). Los inductores tampoco tuvieron un efecto significativo sobre el desarrollo del filamento frontal, el análisis estadístico mostró que el desarrollo del filamento frontal es independiente de los inductores empleados tanto en tejidos de *E. maclovinus* como en tejidos de *O. mykiss*. Este es el primer trabajo que reporta el asentamiento de *C. rogercresseyi* de forma exitosa en sustratos obtenidos desde hospederos silvestres y exóticos, específicamente piel y aleta.

INTRODUCTION

Sea lice (Copepoda, Caligidae), especially *Lepeophtheirus salmonis* (Krøyer, 1837) and *Caligus* spp., have the greatest economic impact of any parasite on salmon fish farming, and they also represent a threat to some wild fish around the world (Costello, 2006). *Caligus royercresseyi* Boxshall & Bravo, 2000, has become the main ectoparasitic copepod affecting salmon and trout farming in Chile since the 1980s (Gonzalez & Carvajal, 1994; Gonzalez et al., 1995; Rozas & Asencio, 2007). This parasite has been transmitted to the farmed fish *Oncorhynchus mykiss* (Walbaum, 1792), *Salmo salar* (Linnaeus, 1758), and *Oncorhynchus kisutch* (Walbaum, 1792) by the native rock cod *Eleginops maclovinus* (Cuvier, 1830) and *Odontesthes regia* (Humboldt, 1833) (cf. Carvajal et al., 1998), thus showing the great ability of the parasite to settle and develop on different hosts, similarly to *Caligus elongatus* (Nordmann, 1832) on the Northern Hemisphere (Pike & Wadsworth, 2000; Kabata, 2003; Johnson et al., 2004). The life cycle of *C. rogercresseyi* has been described in detail by Gonzalez & Carvajal (2003) and it has successfully been completed in the laboratory, both on wild and on farmed fish (Gonzalez et al., 2000; Pino-Marambio et al., 2007). This life cycle, from hatching until the adult stage, varies between 30 and 45 days under temperature and salinity conditions in the ranges of 10-15°C and 25-29 ppt, respectively (Gonzalez & Carvajal, 2003). Eight developmental stages have been established for this parasite: 2 non-feeding and planktonic larvae (nauplius 1, 2), 1 copepodid stage that attaches to a host by its 2nd antennae, and 5 parasitic stages on the host.

Both the settlement process and the selection of a suitable host by copepodids of the family Caligidae are crucial for their individual survival, and there is evidence showing that copepodids (i) respond visually to host shadows and flashes from host scales, (ii) use mechanoreceptors on their antennae to respond to vibrations such as those a host creates, and (iii) use chemoreceptors to respond to water-borne chemical cues (semiochemicals) to recognize hosts (Bron et al., 1993; Heuch &

Karlsen, 1997; Pike & Wadsworth, 2000; Gonzalez & Carvajal, 2003; Bailey et al., 2006). Regarding chemical signals, there are reports showing the preference of copepodids for fish mucus over other host tissues like blood, bile, faeces/urine (Bron et al., 1993). In the salmon lice *Lepeophtheirus salmonis*, conditioned water (CW) of salmon and CW extracts, like isophorone and 1-octen-3-ol, stimulate adult sea lice, and copepodids display high activation and directional responses (Ingvarsdóttir et al., 2002; Bailey et al., 2006). Similar results have been observed in *C. rogercresseyi* when *S. salar* is exposed to conditioned water (CW) (Pino-Marambio et al., 2007). After the suitable host is located, settlement occurs preferably in some areas of the body of the fish (Treasurer & Wadsworth, 2004), as has been reported for *L. salmonis*, which prefers settling on fins (Dawson et al., 1997; Tucker et al., 2000, 2002; Treasurer & Wadsworth, 2004; Genna et al., 2005). Bron et al. (1991) have suggested the lines on fins provide protection from currents and make settlement easier; distribution may be a consequence of current speed and the ability of the copepodid to occupy a given area. We have recently evaluated how *C. rogercresseyi* mainly settles on dorsal and pectoral fins and the caudal fin of *S. salar* (cf. Araya et al., 2011 [unpubl.]). Like other parasitic copepods (Kabata, 1972, cited in Bron et al., 1991), *C. rogercresseyi* settles and fixes itself using a rostral filament (Gonzalez & Carvajal, 2003). Descriptions of filament attachment in parasitic copepods fall roughly into two categories: production of the filament by secretion in situ, or the use of a preformed filament (Kabata, 1972, cited in Bron et al., 1991). The second category has been described for *L. salmonis* (cf. Bron et al., 1991) and *C. rogercresseyi* (cf. Gonzalez & Carvajal, 2003).

This work aimed to experimentally assess the ability of *C. rogercresseyi* copepodids to settle on different tissues of fish, such as fins, scales, and skin extracted from both a wild (*E. maclovinus*) and exotic (*O. mykiss*) host, under laboratory conditions, and to evaluate the effects of inductors, such as conditioned water and fish mucus, on the development of the frontal filament.

MATERIAL AND METHODS

Collection of parasites and copepodids

Approximately 500 ovigerous females of *Caligus rogercresseyi* were collected from farmed rainbow trout (*Oncorhynchus mykiss*) in southern Chile. Parasites were removed from fish previously anesthetized with Benzocaine using fine dissection material and placed on containers with aerated seawater and transported to the laboratory at low temperatures in iceboxes. Once in the lab, ovigerous sacs were removed from the females and cultured in 3 l flasks with artificial seawater (ASW) Instant Ocean Sea Salt (salinity = 32 ppm; temperature = 12°C) in order to allow natural hatching of naupliis. A single pool of larvae was used to evaluate all treatments, which were obtained at day 5 of hatching, when 100% had reached the copepodid stage.

Tissue and inductor preparation for settlement

Tissues of fish used as substrate, and the inductors used to promote settlement were obtained from the natural host species *Eleginops maclovinus* (EM) and the farmed exotic species *O. mykiss* (OM). Besides, fish with a weight ranging between 100 and 200 g were employed to obtain the different tissues used as substrate, which individuals were sacrificed with an overdose of Benzocaine. The following tissues were obtained from each host: skin (SK), fin (FI), and scales (SC); they were washed separately with artificial seawater, dehydrated at 40°C for 2 h, and maintained at −4°C until used. Two inductors were obtained from the same hosts, mucus (MU) and fish-conditioned water (CW), that were applied as inductors directly on the tissue surface immediately after collection. The mucus was extracted from the body surface of the fish using a spatula, while the fish-conditioned water was prepared culturing two fishes from each host for 24 h in 90 L tanks with filtered, UV-sterilized seawater (salinity = 32 ppm; temperature = 12°C). Artificial seawater (ASW) without inductor was used as a control.

Experimental design and data analysis

The experimental assessment of settlement on different tissues (SK, FI, SC) was independently evaluated for each host (EM and OM) and inductor (MU, CW, and ASW). Three replicates per treatment were done, with a total of 9 bioassays per host (3 inductors × 3 replicates). A total of 100 copepodids was added to each replicate (900 per host) with a mean total length of 576 ± 42 μm. Settlement was evaluated in cylindrical flasks with 2.5 L of artificial seawater, low aeration, and in complete darkness (salinity = 32 ppm; temperature = 12°C), where all three tissues (SK, FI, SC) were simultaneously offered. As a settlement substrate, a total of 9 cm^2 was used for each tissue, which was homogeneously distributed in the aquariums through nylon filaments. The number of settled parasites per inductor, substrate, and host was determined under magnification after 24 h. All copepodids (settled and unsettled) were preserved in alcohol 70°, stained with carbol-fuchsin solution, and fixed in Canadian Balsam on a slide. Then, the developmental stage of the frontal filament was classified as early or advanced, using a microscope Nikon S100 according to the description by Piasecki & MacKinnon (1992) for *Caligus elongatus* Von Nordmann, 1832. The category "evaginated" was also considered for those parasites were the frontal filament was everted.

In order to assess the substrate, inductor, and host effect as well as the substrate-inductor interaction, an ANOVA was carried out using the Statistical Analysis System (SAS) software, version 6.12. The following statistical model was used:

$$y_{\mathrm{ijl}} = \mu + H_{\mathrm{i}} + S_{\mathrm{j}} + I_{\mathrm{k}} + I * S_{\mathrm{ij}} + e_{(\mathrm{ijl})}$$

where y_{ijl} is the number of settled copepodids, μ is the total average, H_{i} is the fixed host effect, S_{j} is the fixed substrate effect, I_{k} is the fixed inductor effects, $I * S_{\mathrm{ij}}$ is the fixed effects of the inductor-substrate interaction, and $e_{(\mathrm{ijl})}$ is the residual effect.

A test of independence (Sokal & Rohlf, 1979) was separately done for each host to determine the inductor effect on the development of the frontal filament.

RESULTS

A total of 341 copepodids of *Caligus rogercresseyi* managed to successfully settle on the various tissues, which corresponds to 18.94% of total copepodids used (fig. 1). Regarding the hosts, more copepodids were observed settled on tissues based on the natural host rock cod (N = 215; 23.9%) compared to the exotic host rainbow trout (N = 126; 14%), with significant differences between them (fig. 2; $P < 0.05$). After considering the host effect in the statistical analysis, the tissue showing the higher number of copepodids was the skin (N = 165; 48.4%), followed by fins (N = 140; 41.1%), and scales (N = 36; 10.5%), where significant differences were observed between scales, fins, and skin, although this was not so between skin and scales (fig. 3; $P < 0.05$).

Neither the conditioned water (N = 112; 32.8%) nor mucus (N = 113; 33.1%) showed significant differences with the control without inductor (N = 116; 34%) at the inductor level (fig. 4). As for the frontal filament development, a total of 705 parasites (341 settled and 364 non-settled) were classified according to their developmental stage (table I). The statistical analysis showed the inductors did not have a significant effect on the development of the frontal filament in the tissues based on the wild host rock cod (table I; G = 9.92, g.l. = 20; $P > 0.05$) or the exotic host rainbow trout (table I; G = 15.2, g.l. = 20; $P > 0.05$).

DISCUSSION

This work shows *Caligus rogercresseyi* was able to settle on tissues collected from wild (*Eleginops maclovinus*) and exotic (*Oncorhynchus mykiss*) hosts, thus supporting descriptions on the great capacity of the parasite to settle on different hosts (González et al., 1995; Carvajal et al., 1998; Muñoz & Olmos, 2007; Rozas & Asencio, 2007). However, the larger settlement under artificial conditions (24%)

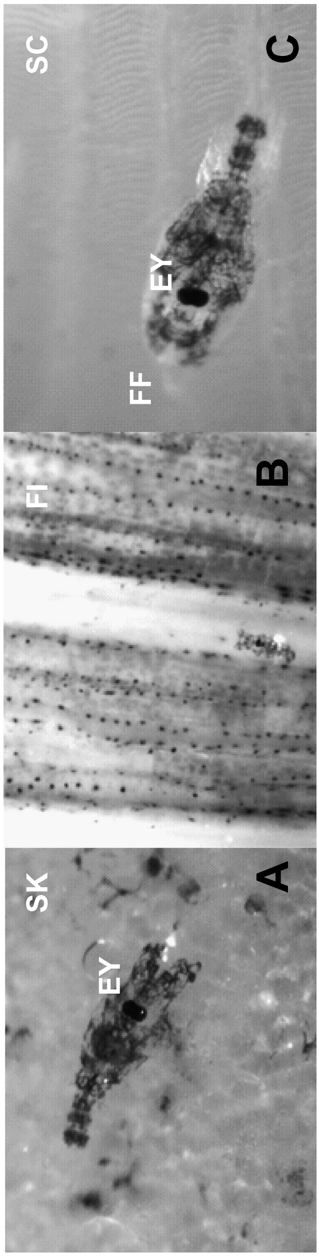

Fig. 1. Copepodids of *Caligus rogercresseyi* Boxshall & Bravo, 2000, settled on the different tissues analysed: A, skin substrate (magnification 6.3×); B, fin substrate (magnification 1×); C, scale substrate (magnification 6.3×). SK, skin; FI, fin; SC, scale; EY, eyes; FF, frontal filament.

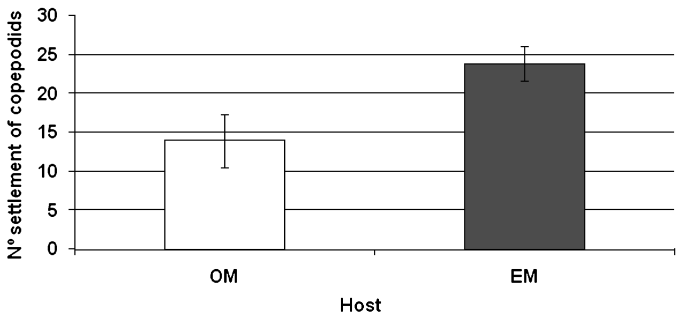

Fig. 2. Mean settled copepodids ± standard deviation per host in the different assessed treatments. OM, *Oncorhynchus mykiss* (Walbaum, 1792); EM, *Eleginops maclovinus* (Cuvier, 1830).

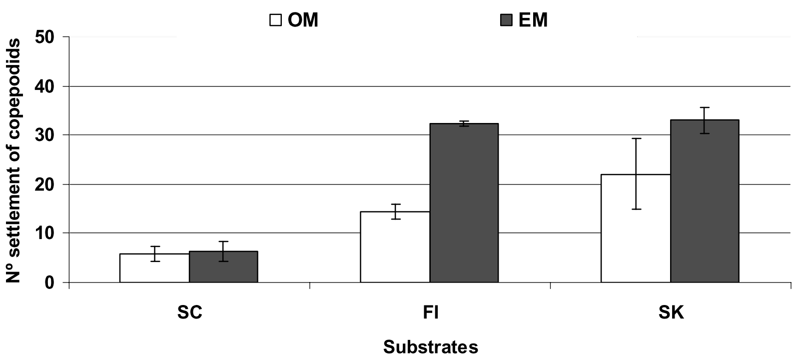

Fig. 3. Mean settled copepodids ± standard deviation for each assessed substrate and host. SC, scale; FI, fin; SK, skin; OM, *Oncorhynchus mykiss* (Walbaum, 1792); EM, *Eleginops maclovinus* (Cuvier, 1830).

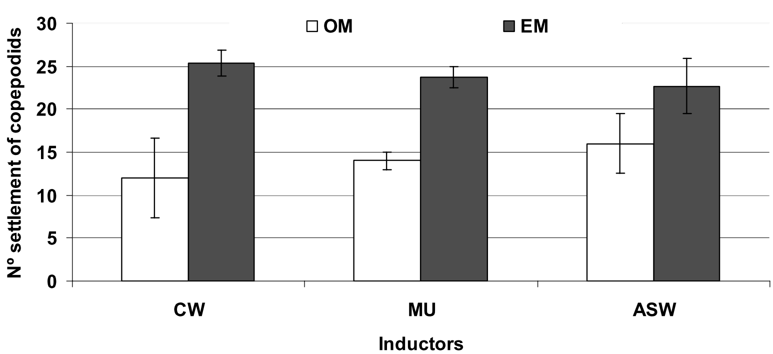

Fig. 4. Mean settled copepodids ± standard deviation for each analysed inductor and host. CW, conditioned water; MU, mucus; ASW, artificial seawater; OM, *Oncorhynchus mykiss* (Walbaum, 1792); EM, *Eleginops maclovinus* (Cuvier, 1830).

TABLE I

Classification of the developmental stages of the frontal filament by host and inductor. Values correspond to the mean ± standard deviation of three replicates and the total number of parasites per treatment

Inductors	*Eleginops maclovinus* (Cuvier, 1830)							*Oncorhynchus mykiss* (Walbaum, 1792)						
	Early		Advanced		Evaginated		Total	Early		Advanced		Evaginated		Total
	mean	X	mean	X	mean	X		mean	X	mean	X	mean	X	
Artificial seawater	1 ± 1.5	4	19 ± 1.5	56	23 ± 3.2	68	128	0 ± 0	0	14 ± 3.1	41	22 ± 2.3	67	108
Mucus	2 ± 1.5	5	18 ± 1.5	55	24 ± 1.2	73	133	2 ± 1.5	5	18 ± 1.5	55	14 ± 1.0	42	102
Conditioned water	0 ± 0	0	16 ± 3.0	48	30 ± 1.2	91	139	0 ± 0	0	14 ± 1.5	41	18 ± 5.0	54	95
Total		9		159		232	400		10		137		163	305

was observed on tissues processed from a natural host, *E. maclovinus* (cf. Carvajal et al., 1998). The settlement percentage recorded for *C. rogercresseyi* on live exotic hosts was 5% for *S. salar*, 12.2% for *Oncorhynchus kisutch*, and 11.25% for *O. mykiss* (cf. González et al., 2000), which is lower than the values obtained in this work under artificial conditions (14%). Gonzaléz (2006) has established that the development of the frontal filament of *C. rogercresseyi* in exotic species, such as *O. mykiss*, results to be slower compared to development on other salmonids (*Salmo salar* and *O. kisutch*) or native species, such as *Hypsoblennius sordidus* (Bennett, 1828). Such effect was not observed in this work, as both rainbow trout and rock cod preferentially showed advanced and evaginated developmental stages of the frontal filament. In relation to the different tissues analysed, the higher preference for settlement on skin and fins may be explained through the fact the parasite is able to hold on more easily with its pair of hooked antennae (González & Carvajal, 2003) than on scales without epithelium. No difference in settlement preference was found between fins and skin, unlike what has been described for the copepodid settlement on live host (Araya et al., 2011 [unpubl.]), maybe because unlike what has been suggested by Bron et al. (1991), the water current was not a limiting factor for settlement in this study. No significant effect was found in this study either for the analysed inductors, or the development of the frontal filament or the settlement success, thus suggesting the tissues used were able to induce a positive response on these processes. This is the first work reporting a successful settlement of a sea lice on tissue extracted from fish.

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