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The spatial distribution patterns of *Caligus rogercresseyi* and *C. elongatus* on Atlantic salmon hosts (*Salmo salar*)

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ABSTRACT

The location of attached and mobile stages of *Caligus rogercresseyi* and *C. elongatus* on Atlantic salmon *Salmo salar* hosts was compared with that of the distribution patterns of *Lepeophtheirus salmonis*. The distribution of *C. rogercresseyi* was also compared in experimental and natural infections, and there were no significant differences for either attached or mobile stages between infection routes. Most *C. rogercresseyi* chalimi were located on the abdomen and post anal areas. Although the distribution of mobile stages was more homogenous there was a significantly higher percentage on the post anal area, 35% of all mobiles, compared with the salmon surface in the post anal region of only 3%. Significantly more attached stages, from 70 to 75%, of both Caligus species were located on the fins compared with mobiles. The mobile stages of both Caligus species had a predilection for the abdominal body. A higher percentage of attached stages of *C. elongatus* was located on the ventral fins and tail compared with *C. rogercresseyi* and, in contrast,significantly more were present on the body in *C. rogercresseyi*. However, there was no difference in the distribution of mobile stages of the two Caligus species with a significantly higher percentage located on the abdomen. In contrast, mobile *L. salmonis* were predominantly located on the back and head. Significantly more attached stages of *L. salmonis* were present on the dorsal fins and adjoining basal epidermis, 30% comparedwith <2% in the Caligus species. These results suggest that *C. rogercresseyi* and *C. elongatus* show similar preferences for the host ventral body and fin locations and there is no direct competition for host substrate between *C. elongatus* and *L. salmonis*.

1. Introduction

While there has been a large focus on the biology of *Lepeophtheirus salmonis*, Caligus species have also had a large economic impact on farmed Atlantic salmon *Salmo salar* (Johnson et al., 2004; Costello, 2009a,b). *Caligus rogercresseyi* in Chile (Bravo, 2003; González and Carvajal, 2003; Bravo et al., 2009) and *C. elongatus* in Europe (Pike, 1989; Revie et al., 2002) and North America (Hogans and Trudeau, 1989) can be a major local and seasonal problem. The reported severity of *C. elongatus* (Bron et al., 1993a) is not as great as that caused by *C. rogercresseyi* in Chile nor with *L. salmonis* in the northern hemisphere (Heuch et al., 2005). The distribution patterns of *L. salmonis* on Atlantic salmon, *S. salar* L., hosts have been well described (Jaworski and Holm, 1992; Pike et al., 1993; Finstad et al., 1994; Dawson et al., 1997; Johnson et al., 2004).

Jaworski and Holm (1992) found that *L. salmonis* had a more anterior distribution in younger salmon than in older fish where there was a variable distribution with many lice in the post-anal region. In experimental infection mobile stages were positioned predominantly anteriorly and dorsally (Pike et al., 1993), with aggregations just behind the head. As intensity of infection increases more mobile stages are found on the ventral and posterior locations (Hull, 1997). The attached stages of *L. salmonis* have a different distribution with most settling at the base of the dorsal fin but with some being found on any part of the body.

In comparison there is little quantitative information about the distribution of mobile and attached stages of *C. elongatus* on salmon and the current information is descriptive (Wootten et al., 1982; Hogans and Trudeau, 1989). Further, there have been no published reports of the distribution of *C. rogercresseyi* on salmonid hosts in Chile. The present work therefore examines and compares the distribution of *C. rogercresseyi* in Chile and *C. elongatus* in Scotland in fish of a similar size range (150–450 g). Both Caligus species show a similar developmental life cycle, comprising 8 stages separated by moults (the planktonic stages nauplius I, nauplius II, copepodid;the attached chalimus I, chalimus II, chalimus III, chalimus IV; and mobile adult). Also, both species are smaller than *L. salmonis*. A length of ca. 5.0 mm and a weight of 3.5 mg have been reported for both sexes of *C. rogercresseyi*(Boxshall and Bravo 2000; Bravo et al., 2010), while for *C. elongatus* a length of 5.4 mm for female and 4.3 mm for male have been reported (Piasecki, 1996). These data are then compared with the distribution patterns of *L. salmonis*, which show important differences with Caligus species. There are 10 stages of development in the life cycle and it has a greater divergence in size. The length of an *L. salmonis* female is 12 mm and it also has a greater

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variation in weight, with an average wet weight of ca. 25 mg compared with 5 mg in the adult male (Bravo et al., 2010). Comparisons are also made of experimental as opposed to natural distribution of *C. rogercresseyi* on salmon hosts.

The rationale for studying the distribution pattern of two Caligus species in different hemispheres is to assess whether different Caligus species show a similar or disparate pattern of distribution. Although there is a different number of developmental stages in Caligus and *Lepeophtheirus* species and the body sizes are different, the life histories with planktonic, attached and mobile stages, are similar. The purpose in studying the distribution in the three species is because quantitative data on body distribution only exist for *L. salmonis* (Jaworski and Holm, 1992), with the favoured location of mobile lice being the head and the anal area. The relevance of studying the distribution of sea lice relates to monitoring surveys, understanding the mechanism of attachment to the host, and also for application in efficacy studies of chemotherapeutants where there may be differential mortality of treated lice.

2. Materials and methods

The following sea lice distributions were compared:

1. The distribution of *C. rogercresseyi* in experimental compared with natural infection.
2. Comparison of attached and mobile lice stages.
3. Comparison of the distribution of *C. rogercresseyi* and *C. elongatus.*
4. Comparison of distribution in *L. salmonis* and the Caligus species.

The methods used for categorisation of body location largely followed Jaworski and Holm (1992) with an amendment that the head and operculum were grouped as one region, the “head”. The dorsal fin was also classified as being part of the anterior back (dorsal) area in the present study rather than splitting the dorsal fin arbitrarily in two and assigning to anterior and posterior back areas. This seemed a more logical grouping and is one used by pharmaceutical and feed companies to assess the efficacy of sea lice medicines (pers. comm., EWOS Ltd) (Fig. 1). Jaworski and Holm (1992)developed a relative infection intensity but this could not be employed in the current study as lice numbers varied between fish on each sampling point, and the current objective was to compare the proportion of lice in each body area rather than numbers. The surface area of each body zone as a percentage of overall salmon surface area follows that of Jaworski and Holm (1992) (Fig. 1).

2.1. Sampling in Chile

Atlantic salmon of 250 g, kept in tanks of 0.5 m^3, supplied with seawater at ambient temperature and salinity, were infected with copepodites produced in vitro, under controlled conditions of temperature (12 °C) and photoperiod (12 h light:12 h darkness), obtained from gravid females collected from Atlantic salmon reared in a farm near Puerto Montt. Fish were sampled 12 days post infestation, anaesthetized with benzocaine (10% in ethanol, 1 ml L^{-1}) and examined macroscopically. The development stage and distribution over the body of ten fish were recorded. Once lice reached the adult stage, fish were also sampled at 26 and 32 days post infestation to record the lice distribution.

In addition, Atlantic salmon of 250 g reared in cages, were monitored using the same criteria as the fish kept in tanks to corroborate the lice distribution under natural infestations. For both cases (experimental and natural infection), a different non identifiable batch of 10 fish was sampled from each unit (tanks and cages) on each date, individually anaesthetized with benzocaine (10% in ethanol, 1 ml L^{-1}), to avoid detaching lice in the water. The parasites were counted by eye and numbers were assigned to body area and recorded by an observer.

2.2. Procedures in Scotland

The fish of 120 to 350 g range in weight were obtained from two sea sites in west Scotland within 6 months of stocking in the sea cages in August and September. The water temperature was in the range 13.5 to 15.8 °C at 4 m depth and salinity varied from 33.2 to 33.9 ppt. Fifteen fish were sampled on each occasion, on two dates, and on two sites (total n = 60 fish). The fish were removed by hand net when the cage net was raised, sacrificed with a blow to the head, labelled and placed in a polythene bag, and transferred to the laboratory in a chilled container and examined within 3 h. The fish were sacrificed on a polystyrene board and lice detached from the head in this process were enumerated and added to the head count, and the implement used was washed and checked to ensure no lice were lost. The fish were examined under a low power (x 40) binocular microscope and all body areas were scrutinised. The fins, including the dorsal and tail fins, were removed from the fish and examined under the microscope separately. Lice, both Caligus species and *L. salmonis* were categorised to copepodid, chalimus(stages I–IV) and mobile stages, and the latter were also identified to sex. Lice were assigned to the body areas as in the survey in Chile, although anterior and posterior areas of the back and abdomen (ventral) surfaces were not distinguished.

2.3. Statistical analyses

Data were expressed as the proportion on each body area of all lice counted on each fish. The data were therefore Arcsine transformed before statistical comparison by ANOVA, Kolmogorov–Smirnov test of overall distribution patterns, and Chi^2 test of the percentage of lice on individual body areas in each inter species and attached/mobile stage comparison. Data were assessed for normality of variances by Bartlett's test and the Arcsine transformation procedure permitted the normalisation of variance of heterogenous data.

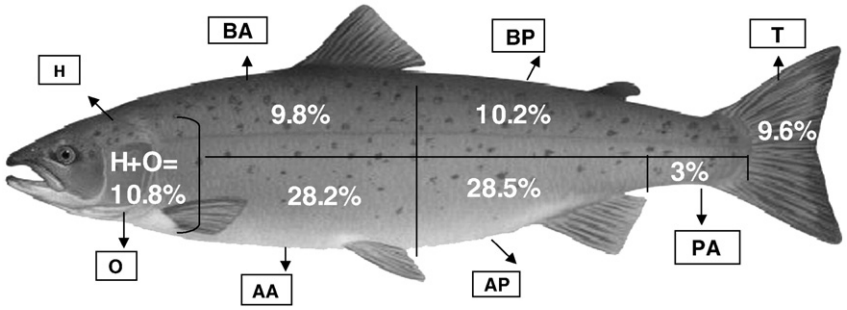

Fig. 1. Body areas of salmon used in the assigning of lice to locational areas, modified after Jaworski and Holm (1992). The epidermal surface area of these zones in salmon as calculated by Jaworski and Holm (1992) is shown to give an idea of the relative importance of each area. H: head; O: operculum; BA: Back anterior; BP: Back posterior; AA: abdomen anterior; AP: abdomen posterior; PA: post anal; and T: Tail.