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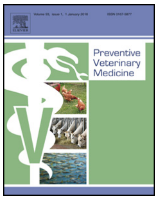

Space–time cluster analysis of sea lice infestation (*Caligus clemensi* and *Lepeophtheirus salmonis*) on wild juvenile Pacific salmon in the Broughton Archipelago of Canada

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ABSTRACT

Sea lice infestation levels on wild chum and pink salmon in the Broughton Archipelago region are known to vary spatially and temporally; however, the locations of areas associated with a high infestation level had not been investigated yet. In the present study, the multivariate spatial scan statistic based on a Poisson model was used to assess spatial clustering of elevated sea lice (*Caligus clemensi* and *Lepeophtheirus salmonis*) infestation levels on wild chum and pink salmon sampled between March and July of 2004 to 2012 in the Broughton Archipelago and Knight Inlet regions of British Columbia, Canada. Three covariates, seine type (beach and purse seining), fish size, and year effect, were used to provide adjustment within the analyses. The analyses were carried out across the five months/datasets and between two fish species to assess the consistency of the identified clusters. Sea lice stages were explored separately for the early life stages (non-motile) and the late life stages of sea lice (motile). Spatial patterns in fish migration were also explored using monthly plots showing the average number of each fish species captured per sampling site. The results revealed three clusters for non-motile *C. clemensi*, two clusters for non-motile *L. salmonis*, and one cluster for the motile stage in each of the sea lice species. In general, the location and timing of clusters detected for both fish species were similar. Early in the season, the clusters of elevated sea lice infestation levels on wild fish are detected in areas closer to the rivers, with decreasing relative risks as the season progresses. Clusters were detected further from the estuaries later in the season, accompanied by increasing relative risks. In addition, the plots for fish migration exhibit similar patterns for both fish species in that, as expected, the juveniles move from the rivers toward the open ocean as the season progresses The identification of space–time clustering of infestation on wild fish from this study can help in targeting investigations of factors associated with these infestations and thereby support the development of more effective sea lice control measures.

1. Introduction

Chum (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*), the most abundant wild salmonid species in the North Pacific Ocean (Noakes and Beamish, 2011), hatch

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from their natal streams and out-migrate into coastal marine waters during the spring and early summer for a saltwater acclimation period in near-shore or estuarine habitats, before heading out to the open ocean later on in the autumn (Heard, 1991; Salo, 1991). During this staging event, these fish can become exposed to sea lice (Morton and Williams, 2003; Jones and Hargreaves, 2007; Gottesfeld et al., 2009).

Sea lice are copepods of the family Caligidae, infesting both farmed and wild salmonids (Boxaspen, 2006; Wagner et al., 2008). The main sea lice species on wild salmon reported on the west coast of Canada are *Caligus* spp. and *Lepeophtheirus salmonis* (Morton and Williams, 2003; Rolston and Proctor, 2003; Wertheimer et al., 2003; Jones and Nemec, 2004; Beamish et al., 2005; Krkošek et al., 2005b; Boxaspen, 2006; Price et al., 2010). Transmission of sea lice occurs when the infective copepodids settle on a host, which is the beginning of the non-motile stage. The sea lice then undergo a moulting process, developing into pre-adult and adult stages (motile stage) that can move freely on the host (Fast, 2014). Through their feeding behavior, these salmon parasites can cause skin erosion, facilitating secondary infections with the opportunistic bacteria of the host (Mustafa, 1997), and increase the susceptibility to and/or risk of viral infection (Petterson et al., 2009; Jakob et al., 2011). Sea lice infestation on chum and pink salmon in the Broughton Archipelago have been shown to be associated with fish length, and to have spatial and temporal variation (Jones and Nemec, 2004; Saksida et al., 2011). The infestation level also varies with different sampling protocols, e.g. seining technique, and live versus lethal sampling (Patanasatienkul et al., 2013; Rees et al., 2015).

Survival of sea lice depends on many factors, including the sea water temperature and salinity. The infective ability and survival of *L. salmonis* copepodids are compromised at the salinity level below 29–30 Practical Salinity Unit (PSU) (Bricknell et al., 2006; Brooks and Stucchi, 2006; Connors et al., 2008). These copepodids may survive at a salinity of 25 PSU; however, the percentage of sea lice developed into copepodids is very low (Johnson and Albright, 1991). Sea-water is progressively diluted when the river freshet occurs during the spring resulting in the reduced salinity along the surface in areas proximate to the river outflows. This creates spatial variation in near-coast salinity levels, possibly causing spatial variation of sea lice, which can be assessed by means of the spatial cluster analysis.

Spatial clustering is a term used to describe the spatial aggregation of some disease, or other event of interest, in a manner that differs from what would be expected simply due to chance. Disease clustering can occur for many reasons such as the infectious spread of disease, the occurrence of disease vectors in a specific location, the clustering of risk factors and the existence of potential health hazards (Pfeiffer et al., 2008). The interplay of the hosts, pathogens together with the compatible environmental condition that could either increase host susceptibility or enhance pathogen infectivity drives the occurrence of a disease (Engering et al., 2013) and their spatial clustering have the impact on disease transmission and the disease control measurement (Tildesley et al., 2010).

Clustering of a disease in space and time can be evaluated using cluster analysis techniques. These analyses can be classified into global and local clustering methods (Pfeiffer et al., 2008). Global clustering methods assess whether clustering exists throughout the study and they measure the degree of spatial clustering without providing the spatial and/or temporal location of the cluster. In contrast, local clustering methods detect the locations of clusters and their extent. Due to the focus of the study in identifying the location of clusters of fish with sea lice infestation, a local method of cluster detection using the scan statistic technique developed by Kulldorff (1997) was applied to this study. The space–time scan statistic is a commonly used method for detection of clusters of disease or some other event of interest in space and time. The analysis can be performed through freely available software: SaTScan^{TM} (Kulldorff, 2011).

The scan statistic technique has been applied in various areas of study, including human and animal epidemiological research (Carpenter, 2001). The scan statistic has been widely used in the human health, for examples, to detect cluster areas with high breast cancer mortality in the United States (Kulldorff et al., 1997), to study spatiotemporal clusters of tuberculosis in Portugal (Nunes, 2007), and to identify hot-spots of malaria transmission and predict future infection (Mosha et al., 2014). In animal health, this method has been applied mostly in disease surveillance of terrestrial animal populations. Norström et al. (2000) detected clusters of acute respiratory disease in cattle using the space–time scan statistic. The space–time permutation model was applied to assess spatial and temporal pattern of Rift Valley fever outbreaks in humans and domestic ruminants (Sindato et al., 2014). Although this method has become familiar to animal health researchers, there are still very few applications of the scan statistic technique in an aquatic context. It has been used to assess clustering of salmonids that had antibody against viral hemorrhagic septicemia virus in Switzerland (Knuesel et al., 2003), to detect low/high risk area of *Toxoplasma gondii* infections in southern sea otters (Miller et al., 2002), and to detect space–time cluster of infectious salmon anemia virus cases in Chilean Atlantic salmon farms (Godoy et al., 2013). All of these studies assumed Euclidean distance between locations and, therefore, may have misrepresented true distances. The mechanisms of disease transmission in aquatic environments typically differ from those in terrestrial animals. In an aquatic environment, transmission between any two locations usually occurs through the water body and the seaway-distance is an appropriate measure for quantifying the distance. In river networks or complex coastal inlet geographic situations, non-Euclidean distances will have to be used, which can be accommodated in SaTScan^{TM} when defining the size of the scanning window.

Analyzing surveillance data can be a challenging task for a number of reasons, including missing data and the need to use multiple datasets. The univariate scan statistic tests whether there is an increased or decreased risk of an event of interest within, compared to outside, a typically circular area in a single dataset (Kulldorff, 1997). To be able to allow combined analysis of multiple datasets with each representing different host populations or time periods,