Beaver wetland and lake effects on *Francisella tularensis* in Swedish landscapes and hares

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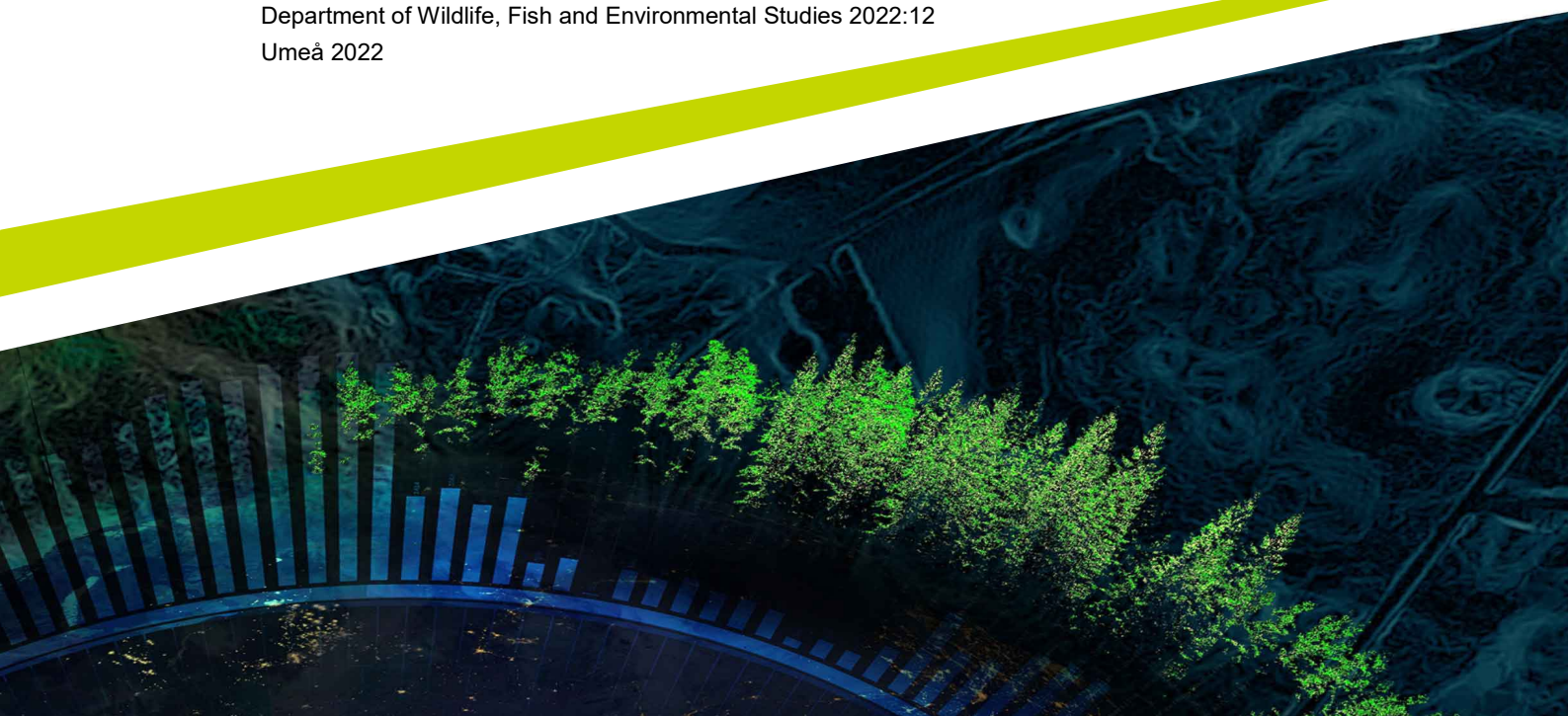

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Abstract

The zoonotic disease tularemia is caused by the bacterium *Francisella tularensis* that infects both humans and wildlife in Sweden. Associations between outbreaks of tularemia and lakes and rivers have earlier been observed, and semi-aquatic rodents and mosquitoes are believed to be key species groups in the epidemiological cycle of the disease. I have conducted a series of landscape analyses to assess land cover properties with focus on water availability at different spatial scales in areas of tularemia incidence, using data on hares found dead (n= 452) with known tularemia status, collected in 2016-2021, and land cover properties. As a complement, 56 water samples (biofilm and surface water) from lakes and beaver ponds in Sweden, covering latitudes from N 59° 29.0582' in the south, to N 65° 52.4261' in the north, were collected to investigate the occurrence of tularemia in different freshwater ecosystems. Results show significant correlations between tularemia occurrence and high soil moisture, and local proportion of inland water area. Water proximity as well as proportional wetland area both lack significant effects on tularemia status in hares. In water samples, I found *F. tularensis* in 11% of 56 samples, which were all taken from beaver systems. No difference in sampling depth could be found as *F. tularensis* was found in both biofilm and surface water. I suggest further investigations on *F. tularensis* in wetlands to a) assess the role of water biogeochemistry including pH, turbidity, and nutrient levels for occurrence and environmental persistence of the bacterium and b) evaluate the role of beaver systems for the epizootiology and epidemiology of tularemia with beavers as either reservoirs and/or beaver systems favoring vector (mosquito) habitat.

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1. Introduction

1.1 Tularemia and its epidemiology

Tularemia is a zoonotic disease with a geographical range covering most of the northern hemisphere (Sjöstedt 2007) with outbreak hotspots in central continental Europe and Scandinavia (Hestvik et al. 2015), with up to 817 confirmed human cases per year in Sweden alone (European Centre for Disease Prevention and Control 2021). The causative pathogen of tularemia is *Francisella tularensis*, a gram-negative bacterium with four recognized subspecies: *F. t. tularensis, holarctica, mediasiatica* and *novicida* (Maurin & Gyuranecz 2016). It is mainly transmitted via contact with infected animals or vectors such as mosquitos and ticks (Dahlstrand et al. 1971; Černý 2001; Sjöstedt 2007; Tärnvik & World Health Organization 2007; Carvalho et al. 2014; Hestvik et al. 2015), but consumption of contaminated water, crops, or food (Dahlstrand et al. 1971; Friend 2006; Brantsaeter et al. 2007; European Centre for Disease Prevention and Control 2011a) and inhalation of bacterial aerosols are alternate pathways of transmission (Stewart 1996; Tärnvik et al. 2004; Friend 2006; Delaney et al. 2018). After transmission, the bacterium attacks its host's internal organs (Delaney et al. 2018) which is fatal to many animals, including lagomorphs and rodents (Sjöstedt 2007; Hestvik et al. 2017; Delaney et al. 2018).

Francisella tularensis tularensis, often referred to as *type A*, is the most virulent subspecies and is predominantly found in North America (Carvalho et al. 2014). Subspecies *holarctica*, or *type B*, causes a milder form of tularemia in humans, and has both a terrestrial cycle, with ticks as a central vector (Mörner 1992; Carvalho et al. 2014; Maurin & Gyuranecz 2016), and a waterborne cycle including aquatic rodents such as beavers (*Castor fiber* in Eurasia and *C. canadensis* in North America; Mörner et al. 1988b; Mörner 1992; Friend 2006; Kevin et al. 2021), with mosquitos being the main vector infecting both animals and humans (fig. 1, Eliasson et al. 2002; Rossow et al. 2014b; Maurin & Gyuranecz 2016; Hestvik et al. 2017). In Scandinavia, the waterborne cycle dominates (Mörner 1992; Desvars et al. 2015; Hestvik et al. 2017). Both the terrestrial and waterborne cycles of type B use hares as a main host (Mörner 1992; Sjöstedt 2007).

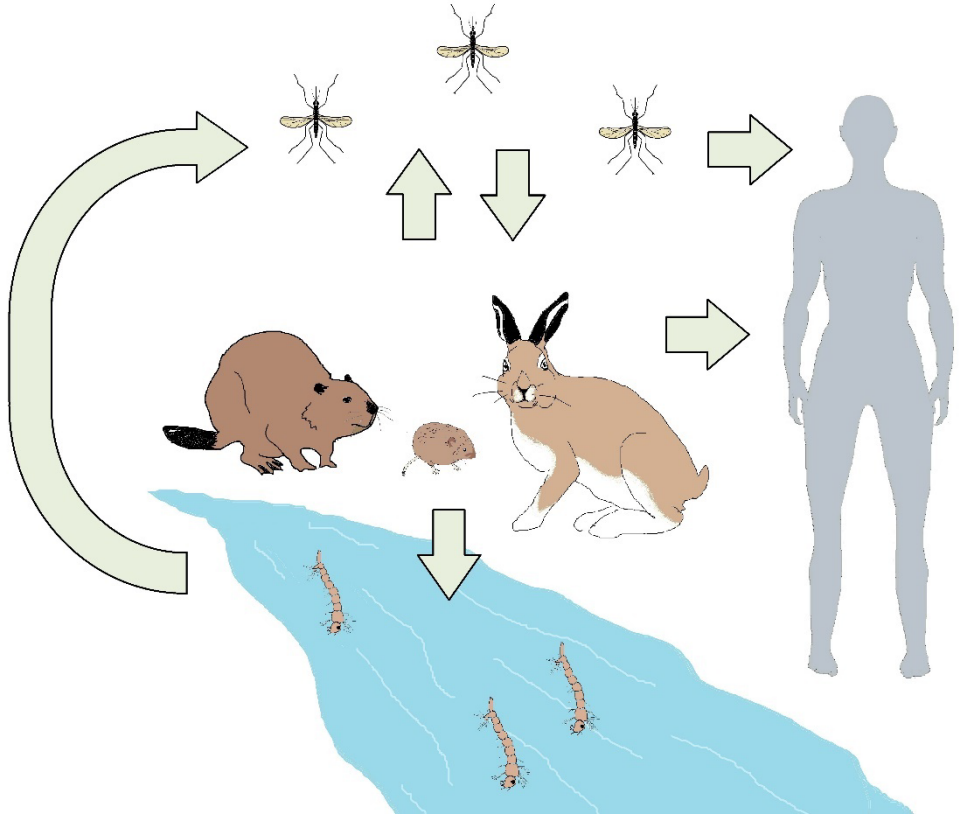


Figure 1. Schematic illustration of the waterborne cycle of tularemia. The mosquito larvae obtain the bacterium through the water they inhabit. When adult, they can infect both animals and humans when sucking blood, they can also get infected if they feed on infected animals. Animals can infect humans via direct contact. The bacterium spreads from infected animals to surrounding waters via excrement and deceased carcasses.

F. tularensis has been found in at least 190 mammals, 88 invertebrates, 23 birds, three amphibians and a few species of reptiles and fish (Mörner & Addison 2001; Friend 2006; Decors et al. 2011), giving the bacterium the broadest host range of zoonotic disease-causing organisms known (Broman et al. 2011; Gyuranecz 2012:22). Sensitivity to infection varies highly with species (Mörner & Sandstedt 1983). Cases of interhuman transmissions have not been reported and are, if not impossible, extremely rare (Sjöstedt 2007). Animals that do not develop acute disease, but can persist harboring the bacterium, may act as reservoirs for *F. tularensis* (Hestvik et al. 2015). Infection of hares and other animals is probably similar to that of humans, i.e. consumption of contaminated water or food (Gyuranecz 2012:22), inhalation of aerosols or arthropod bites (Decors et al. 2011), where the latter is thought to be of particular importance (Hestvik et al. 2017). Small rodents, beavers and lagomorphs are amplifying hosts, i.e. hosts in which bacteria can replicate to high levels for further contamination, and an important source for human infections of tularemia (Tärnvik & World Health Organization 2007; Decors et al. 2011; Gyuranecz et al. 2011; Rossow et al.

2014a), and probably the key reservoir animals (Gyuranecz et al. 2011; Rubin 2018). In Scandinavia, most important species are the European brown hare (*Lepus europaeus*) and the mountain hare (*L. timidus*, National Veterinary Institute - SVA 2020).

Death in humans caused by tularemia in Europe is extremely rare, and the infection is treated with antibiotics (Sjöstedt 2007). In USA, on the other hand, the same ssp. *holarctica* had as late as in 2009 a mortality rate of 7% (Kugeler et al. 2009). Outdoor activities, farming and hunting are the major risk exposures to *F. tularensis* (Dahlstrand et al. 1971; Stewart 1996; Černý 2001; Tärnvik & World Health Organization 2007; Moinet et al. 2016). Human incidences show a clear pattern of seasonality in Europe (Černý 2001; European Centre for Disease Prevention and Control 2011a; Carvalho et al. 2014; Maurin & Gyuranecz 2016). This is however not visible in animals; one possible explanations for this is the peak in outdoor activities and hence the contact with sources of tularemia (Hestvik et al. 2015).

Links to the multitude of tularemia have been shown to be war and economy (Tärnvik et al. 2004; Friend 2006; Sjöstedt 2007) which gives rise to the question why Sweden, despite economical welfare has been among the most affected of tularemia in Europe the past decades (Sjöstedt 2007). Occurrence of *F. tularensis* in the environment does not necessarily equal the incidence and spread of tularemia (Tärnvik et al. 1996; Sjöstedt 2007; Hestvik et al. 2015). Sporadic occurrence of tularemia cases far from endemic areas suggests that the bacterium is wider spread than the region of tularemia outbreaks; epidemiological circumstances must be in place for the disease to spread and outbreaks of tularemia to occur (Tärnvik et al. 1996, 2004). Such circumstances may be ecological, climatic and environmental (Mörner 1992; Hestvik et al. 2015). The virulence of *F. tularensis*, the availability of feasible hosts and vectors, and their abundance and resistance to infection also play a major role in the magnitude of tularemia outbreaks (Mörner 1992; Hestvik et al. 2015). It is hence important not to solely focus on one link in the ecological chain of tularemia but to investigate mammalian hosts, vectors and their surroundings (Sjöstedt 2007). Mörner (1992) lists climate as an important factor influencing the ecology of tularemia, and a projection of 2100 climate by Rydén et al. (2009) suggests that tularemia is likely to increase in the future. The seemingly temperature induced season for human outbreaks, triggered by vector activity, may expand as much as twofold with higher temperatures, despite conservative projections (Rydén et al. 2009). In a more recent study, Ma et al. (2020) suggest an increase in tularemia in northern and central Sweden, but a decrease in southern parts of the county. Yet, they stress the difficulty to make reliable projections as an elevated temperature tends to increase mosquito biting rate, alongside with an increased mosquito mortality (Rohr et al. 2011; Ma et al. 2020). Buffering effects from global warming may

occur as *F. tularensis* is heat and light sensitive, whilst low temperatures favor bacterium survival (Friend 2006; Gyuranecz 2014).

1.2 Tularemia in Sweden

The most frequent hosts of tularemia in Scandinavia is the mountain hare, European brown hare and small rodents (Mörner 1992; Hestvik et al. 2017; National Veterinary Institute - SVA 2020), probably due to their high sensitivity to the bacterium (Tärnvik & World Health Organization 2007). The waterborne cycle with mosquitoes as a main vector dominates in both Sweden and Finland, (Ekdahl & Twisselmann 2001; European Centre for Disease Prevention and Control 2011b; Desvars et al. 2015) and transmission to both animals and humans predominantly occurs via mosquito bites (Dahlstrand et al. 1971; Mörner et al. 1988b; Eliasson et al. 2002; European Centre for Disease Prevention and Control 2011a; Hestvik et al. 2017).

In Sweden and in Europe in general, there is a strong correlation between the epizootics and peaks in reported human infections (Mörner & Sandstedt 1983; Mörner 1992). Also, peaks in Swedish vole and mountain hare population sizes happen to coincide with an increase in reported human cases of tularemia (Dahlstrand et al. 1971; Mörner & Sandstedt 1983; Hörnfeldt et al. 1986; Tärnvik et al. 1996; Friend 2006). However, these correlations are not spatially nor temporally consistent, as pronounced cyclic behavior of northern vole populations occurs alongside with few cases of human tularemia (Tärnvik et al. 1996).

The northern part of central, western Sweden is hotspot for tularemia, having a historical stronghold where the provinces of Gästrikland, Hälsingland and Dalarna meet (Dahlstrand et al. 1971; Mörner & Sandstedt 1983; Tärnvik et al. 1996; Eliasson et al. 2002; Folkhälsomyndigheten 2021), with most cases occurring between July and September (Dahlstrand et al. 1971). Looking at reports between 2005 and 2010, the trend is an expansion of tularemia in Sweden, both concerning geographical range and number of cases reported (Hestvik et al. 2015). Historically, southern Sweden has only had very occasional cases of tularemia (Tärnvik et al. 1996, 2004), but in the past few decades, the epidemic has expanded southwards (Rydén et al. 2009; Folkhälsomyndigheten 2021).

1.3 Tularemia in hares

Hares belong to the order lagomorphs that together with rodents is the most suffering order from tularemia (Ahangari Cohan et al. 2020; National Veterinary Institute - SVA 2020). In Europe, hares are key-species with regards to tularemia and its epidemiology (Mörner et al. 1988b; Gyuranecz 2012; Hestvik et al. 2017; National Veterinary Institute - SVA 2020). In Sweden there are two species of hare: The native mountain hare, and the introduced European brown hare. Both

species can develop subacute or acute tularemia (Gyuranecz et al. 2010; Decors et al. 2011). The hares suffering from subacute tularemia develop chronic lesions and can live with the bacteria for long periods of time (Hestvik et al. 2015) and hence efficiently act as reservoir hosts (Gyuranecz 2012). Hares developing acute disease die from acute infection in their liver, spleen, and bone marrow within approximately five days after infection (Mörner & Sandstedt 1983; Mörner et al. 1988b; Mörner 1992; Hestvik et al. 2017).

As hares are highly sensitive to tularemia, they act as an *early warning* in an upcoming epidemic (Hestvik et al. 2015): In tularemia outbreaks in Scandinavia, mountain hares are usually the first animal to be found sick, and it only takes weeks before regional populations decimate (Berdal 1996).

1.4 Hare ecology and conservation status

Lagomorphs and rodents play an important role in the ecosystem as they make up a large portion of many predators' diet (Strand et al. 1999; Odden et al. 2006; Andrén & Liberg 2008; Schneider & Sahlén 2018; Thulin et al. 2021). Hence studies of the wellbeing of hares can be important in an ecosystem point of view.

Italy, France, UK and Sweden all have the mountain hare red listed in terms of range and/or population growth (European Environment Information and Observation Network 2018), and in southern Sweden, the endemic subspecies *L. timidus sylvaticus* faces risk of extinction (Thulin et al. 2021). Diseases such as tularemia, competitive exclusion and hybridization with the European brown hare are factors detrimental for the native mountain hare (Wilson et al. 2017). Observed declines in European brown hare populations are mainly explained by habitat degradation (Pavliska et al. 2018).

Home range of the mountain hare differs with habitat and climate, but in the boreal coniferous forests of Scandinavia, it is approximately 200 ha (Wilson et al. 2017). For the European brown hare, typically living in a more open, agricultural landscape, home ranges span between approximately 20 ha in the southern Sweden to considerably larger 140 ha in the central parts of the country (Jansson & Pehrson 2005). Landscape use differs between the two species, where the European brown hare mainly is found in the open landscape such as agricultural land where it forages on grasses and herbs (Jansson & Pehrson 2007). The mountain hare, on the other hand, prefers dwarf and berry shrubs, twigs, buds and young, deciduous bushes (Dahl 2005; Jansson & Pehrson 2007)

1.5 Tularemia in beavers and wetlands

Other than lagomorphs, rodents such as voles, lemmings and beavers are important hosts of *F. tularensis* in its aquatic cycle, where the latter is sporadically associated with human cases of tularemia (Forsman et al. 2000;

Maurin & Gyuranecz 2016). Infected and diseased beavers spread the bacterium during removal, skinning and capture of animals, and through the water in their ponds (Jellison et al. 1942; Friend 2006; Schulze et al. 2016; Yapar et al. 2016). This as urine, excrement and carcasses from infected beavers contain live bacteria, which may contaminate surface water and hence be a source of further transmission (fig. 1, Jellison et al. 1942; Friend 2006; Gyuranecz 2012; Schulze et al. 2016; Yapar et al. 2016). High levels of *F. tularensis* have been observed in surface water where beavers have been infected (Jellison et al. 1942) and it has been suggested that beavers together with lemmings could be reservoir hosts in water (Constable et al. 2017; Rubin 2018). In 1940, Jellison et al. found that *F. t. tularensis*, given low temperatures, could survive in the mud and water of beaver ponds up to 33 days after infected and diseased beavers had been removed. Eurasian beavers (*Castor fiber*) are not far as prone to death by tularemia as the American beaver (*Castor canadensis*, Jellison et al. 1942; Mörner & Sandstedt 1983; Payne 1989; Petrosyan et al. 2019): In a study performed by Mörner and Sandstedt (1983), antibodies for tularemia were found in 63 of 110 (57%) tested Swedish beavers. This finding indicates resistance to the bacterium and supports the idea of beavers as a reservoir host (Mörner & Sandstedt 1983; Mörner et al. 1988a; Rubin 2018). In contrast, American beavers *(Castor canadensis)* are extraordinarily susceptible to tularemia (Petrosyan et al. 2019) and typically die quickly after being infected (Jellison et al. 1942; Tärnvik & World Health Organization 2007); this despite them being affected by the same bacterium, i.e. type B, as beavers of the Old World (Constable et al. 2017). In 1940, up to 200 American beavers were found dead in the state of Montana, and out of 10 animals tested for tularemia, all were positive. Beavers in this region were not harboring ticks nor other blood-sucking arthropods, and during the season of this epizootic (November to January) no mosquitoes, deerflies or other possible insect vectors are active. Hence, Jellison et al. (1942) concluded that water was the most probable route of transmission, and that lodges built in stagnant water brought a higher risk of infection in beavers compared to constructions in flowing water.

In Sweden, beavers were extinct in the late 19th century, but rewilding projects across Europe reimplanted Norwegian beavers that successfully recolonized the Scandinavian peninsula (Hartman 1994). The current population size is estimated to approximately 100,000-150,000 animals in Sweden alone (Hartman 1995; Sjöberg et al. 2019). The reintroduction of beavers may increase the occurrence of wetlands as beavers' dam constructions create these environments (Cunningham et al. 2006; Ecke et al. 2017; Willby et al. 2018).

F. t. holarctica is associated with ponds, rivers and lakes (Forsman et al. 2000; Svensson et al. 2009; Desvars et al. 2015; Constable et al. 2017); habitats typically associated with beavers. This has been observed across the biogeographic region the bacterium (Hestvik et al. 2015; Maurin & Gyuranecz 2016) and it has been found in free waters such as lakes, streams and ponds

(Jellison et al. 1942; Broman et al. 2011; Ahangari Cohan et al. 2020). *F. tularensis* appears to survive in water outside of its vectors and hosts for months (Gyuranecz 2014), and surface water is considered to be an important route for transmission (Ahangari Cohan et al. 2020). The survival of *F. tularensis* in water is not fully elucidated but nutrient rich environments in coexistence with predating protozoa have been shown to help the bacterium survive and multiply (Thelaus et al. 2009). Replication of *F. tularensis* in protozoa has been observed in experimental settings (Berdal 1996; Abd et al. 2003; Tärnvik et al. 2004) and protozoan reservoirs have been observed in other facultative intracellular bacteria (bacteria living and reproducing in the cells of a host, Tärnvik et al. 2004). Over time however, *F. t. holarctica* loses its virulence in water despite high levels of protozoa and nutrients (Thelaus et al. 2009).

1.6 Mosquitoes and wetlands

The seasonality of Swedish peaks in human infection overlaps with the mosquito season, June to September, which may indicate a correlation (Hestvik et al. 2015). Human incidences have been shown to be higher in areas near lakes, streams, ponds and rivers (Sjöstedt 2007; Svensson et al. 2009; Desvars et al. 2015) and in summers with more precipitation (Ekdahl & Twisselmann 2001). Likely due to the environmental factors suitable for the bacterium's waterborne cycle being in place. Mosquitoes are dependent on water bodies for their reproduction (Rossow et al. 2014a; Chandrasegaran et al. 2020); wet, shallow environments may hence favor the abundance of tularemia (Rydén et al. 2009) as mosquitoes are the main vector of the disease in hares and humans in Sweden (Mörner 1992; Tärnvik et al. 1996; Hestvik et al. 2017).

Mosquitoes feed on protozoa during their larval stage, which is thought to be the transmission route from water to vector (Rydén et al. 2009; Lundström et al. 2011). Adult mosquitoes can obtain the bacterium if they feed on infected animals (fig. 1, Thelaus et al. 2014). This raises the question about potential consequences from wetland restoration, where biological diversity, storage of greenhouse gases and haltering of wildfires are some of the motivators for turning drained wetlands back into their natural shape (Eiseltová 2010; Law et al. 2017; Willby et al. 2018). This has been a response to degradation and drainage of wetlands across the planet (Willby et al. 2018), and Sweden is no exception (Gren 1995; Willby et al. 2018). Rydén et al. (2009) claim that an increase in nutrient-rich wetlands in proximity to humans may increase incidence and magnitude of human tularemia cases. Hence, the human restoration of wetlands may have a knock-on effect on tularemia outbreaks (Desvars et al. 2015).

Mosquitoes prefer forested areas in similarity to many of their blood-meal hosts, and lay their eggs in waters with minimum predation, e.g., temporary wetlands or even directly on the soil, that later may be submerged during floods

(Schäfer et al. 2006). Presence of temporary waterbodies and wetlands have thus been shown to favor mosquito abundance in Sweden (Schäfer et al. 2006). Schäfer et al. (2006) suggest that permanent wetlands in open landscapes may reduce mosquito colonization, and instead favor mosquito predators.

Mosquitoes are generally more abundant in central and northern Sweden compared to southern parts of the country (Schäfer et al. 2006; Schäfer & Lundström 2009; Lundström et al. 2013), but since the turn of the millennium, large numbers of mosquitoes have been observed in southern Sweden (Schäfer & Lundström 2009). Abundance and range are predicted to increase further as annual precipitation is projected to increase with the ongoing climate change, especially in the north (Lindgren & Jaenson 2006; Lundström et al. 2011).

1.7 Aims of this study

Given the importance of hares, beavers, and wetlands in the epidemiology of tularemia, I used data on hares, found dead and tested for tularemia, in combination with landscape analyses as well as field and laboratory studies to investigate the following research questions:

1. Does wetland and inland water availability increase the risk of tularemia infection in hares found dead?
2. Is there an increased risk of finding *F. tularensis* in water associated with beavers, compared to lakes?

2. Methods

Analyzing land cover, I had variables for wetlands and inland water (lakes and watercourses) together with different forest types and exploited areas. Using logistic regression models, I compared patterns in soil moisture and land cover around hares testing positive and negative for tularemia. This within three sets of 452 estimated home ranges of hares in Sweden, all corresponding to sites where dead hares had been found. Each set corresponds to the range sizes of 500 m, 1300 m, or 1600 m in diameter, relating to estimated min, mean and max sizes of hares home ranges (Jansson & Pehrson 2005; Wilson et al. 2017). I also compared the two hare groups regarding latitude, longitude, and the distance between the hares and closest inland water and wetland.

I hypothesized that the variables *wetland* and *inland water* would have a higher coverage in areas associated with tularemia as water accessibility favors mosquito vectors and spread of the bacterium (Jellison et al. 1942; Berdal 1996; Gyuranecz 2012; Rydén et al. 2012; Balci et al. 2014; Desvars et al. 2015). I also hypothesized that higher soil moisture would correspond to an increased risk of hares testing positive for tularemia.

In addition, I sampled and tested the water in lakes and beaver systems adjacent to sites of hares found dead with tularemia, looking for patterns in sample depth and water type. The method used is not established and should thus be viewed as a pilot study. With these results I wish to contribute to further knowledge about tularemia geography and how wetland restauration may impact spread of the disease. All equipment is listed in appendix 1.

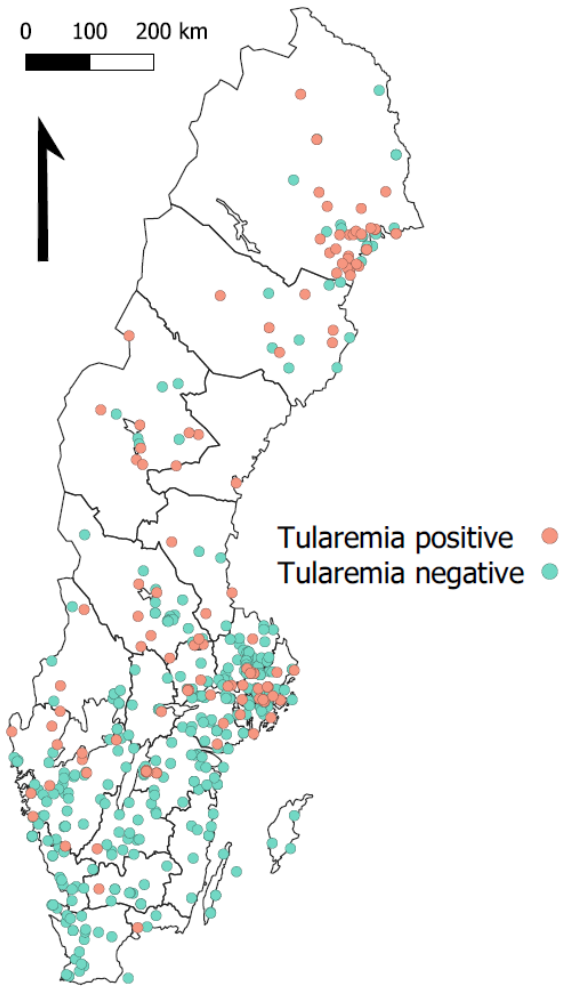
2.1 Landscape analyses

2.1.1 Dataset on hares

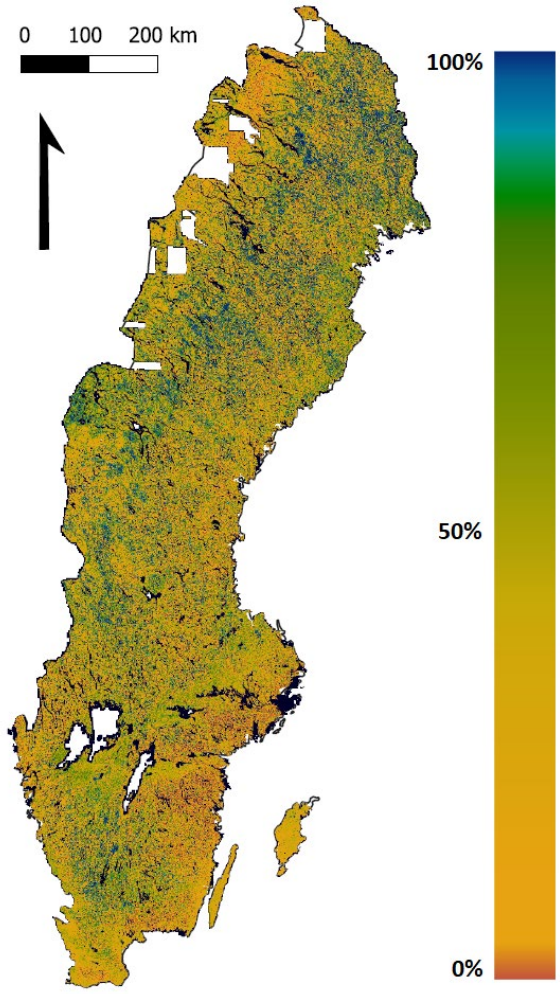
Deceased hares found in the wild by the public have been reported and handed in to the National Veterinary Institute (SVA) where they were tested for tularemia (SVA 2021). Data on hares used in this study was provided by the SVA. The dataset included coordinates of sites where hares have been found dead and tularemia status for each hare (i.e., if a hare tested positive (ft+) or negative (ft-) for tularemia, respectively), covering a timeseries from January 2016 to May 2021 (fig. 2 A). The data included 115 hares (52 European brown hares and 63 mountain hares) that tested positive for tularemia, and 337 that tested negative.

2.1.2 Landcover analyses

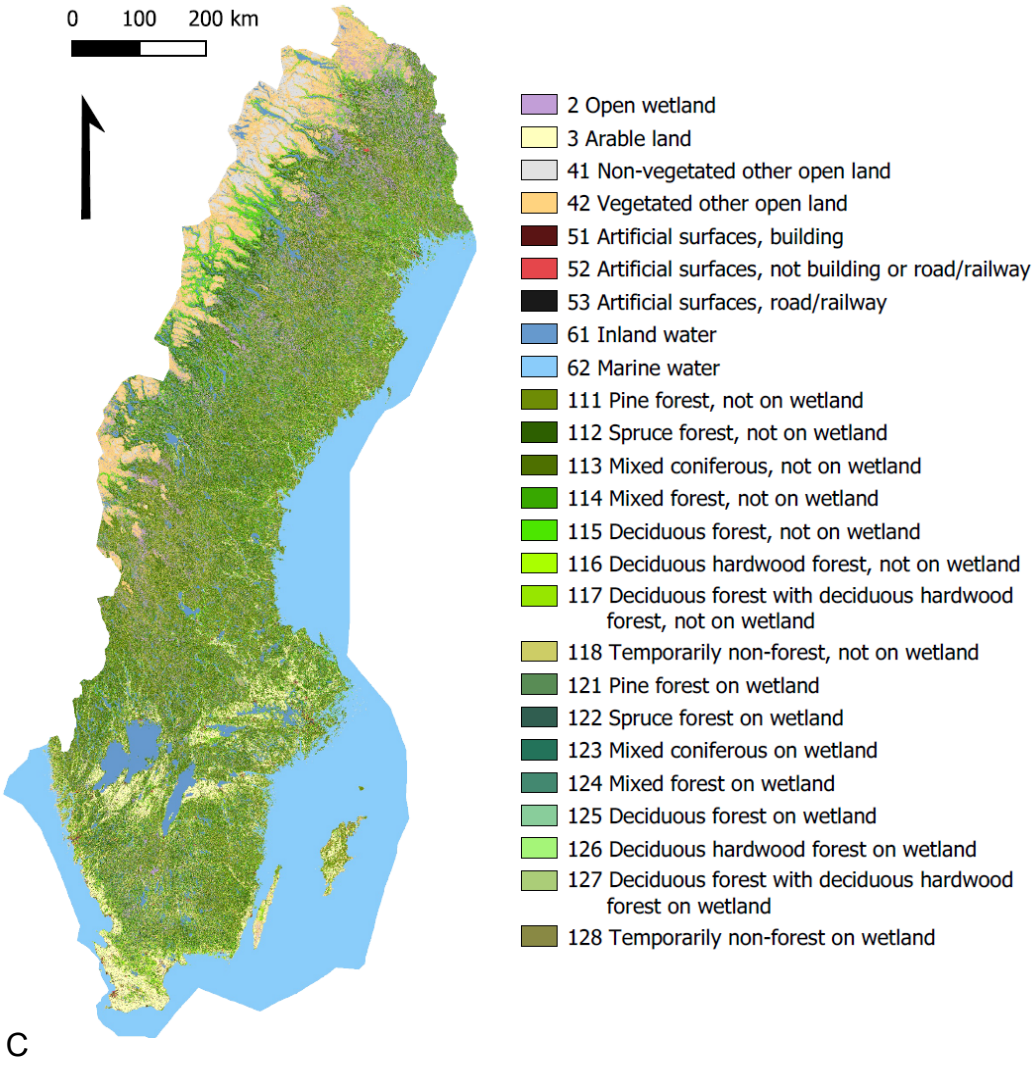
To detect patterns in environmental factors coinciding with confirmed tularemia in hares, I conducted statistical analyses using software QGIS and RStudio (QGIS *version 3.16.3*, 2021, RStudio *version 2021.9.0.351*, 2021). I used environmental data on *soil moisture* (fig. 2 B) provided by Swedish University of Agricultural Sciences (Ågren & Lidberg 2020; Ågren et al. 2021) and *land cover* (fig. 2 C) from the National Land Cover Database by the Swedish Environmental Protection Agency (Swedish Environmental Protection Agency 2021). I used QGIS to create three sets of buffer zones around each coordinate where hares had been found. The first set had a buffer zone diameter of 500 m, the second a diameter of 1300 m, and the third a diameter of 1600 m. These sizes were set to reflect the span of hares' home ranges (Jansson & Pehrson 2005; Wilson et al. 2017). This approach relies on the assumption that each hare was found in the very center of its home range. Raster files were extracted from layers *soil moisture* and *land cover*, and divided into two groups: Tularemia positive and tularemia negative hares. The two groups of buffer zones associated with tularemia positive, and tularemia negative hares, respectively, were then analyzed in RStudio using *raster* and *tiff* packages. I calculated the individual coverage of each land cover type (e.g., mire, pine forest, lake etc.) and the mean soil moisture for each of the zones. The three variables describing *artificial surfaces* (buildings, roads/railways, and not building or road/railway) were merged into one class, and land cover variables with a mean coverage of <2% were removed from the analyses.


0 100 200 km
Tularemia positive
Tularemia negative

A


0 100 200 km
100%
50%
0%

B



Figures 2 A-C. A: Spatial datasets used to analyze the relationship between tularemia in hare and landscape properties. Locations of hares reported to, and tested for tularemia by the SVA between January 2016 and May 2021. Black lines are county borders. B: Soil moisture map over Sweden, with continuous values from 0-100% moisture, SLU 2020. C: Land cover map over Sweden, provided by the Swedish Environmental Protection Agency, 2020.

Variables *vegetated other open land*, *arable land* and *artificial surfaces* were all removed due to high correlations with other variables and high VIF (variance inflation factor) values. The variables describing forests on wetland (121-128) were removed from the analysis due to too little coverage for meaningful analysis. Eleven logistic regression models with a varied composition of the 12 remaining variables (y-variable being tularemia status) were tested based on the AUC (area under ROC curve), aggregated means over all variables, the corresponding y-value when the curves for the model's sensitivity and specificity intersect, and the accuracy of the model's resulting predictions when minimum TPR (true positive rate) was set to 10. The model with the best performance was a

model containing eight of the variables for land cover and soil moisture, including *wetland*, *inland water*, *pine forest*, *spruce forest*, *mixed forest*, *deciduous forest*, *deciduous hardwood forest* and *soil moisture*. This was the case for all three buffer zone sizes.

The three ultimate logistic regression models, one per buffer size, were then run to detect statistical differences between ft+ and ft- buffer zones. This to determine if environmental variables could predict or explain the occurrence of tularemia in hares. As the final models contained 8 variables each, I used Bonferroni-correction resulting in a corrected significant p-value limit of 0.05/8 = 0.00625.

McFadden's R^2 was computed as:

$$R^2 = 1 - \frac{Residual\ deviance}{Null\ deviance}$$

(University of Illinois 2016)

2.1.3 Locational analyses

Three conditional density plots and three boxplots were drawn, one for each buffer size to visualize the change in tularemia probability with higher soil moisture.

In addition, distances between each hare coordinate and closest (1) wetland and (2) inland water (lake or watercourse) with an area of ≥100 m^2 were calculated using NNJoin plugin in QGIS (Tveite 2022). The distances to these two landcover types together with the coordinates of the hares were then compared between hares with, and without tularemia, respectively, using logistic regression. This to detect if ft+ hares were in closer proximity to inland waters and wetlands, and if there was any pattern regarding longitude and latitude. Outliers (>11,000 m) were removed as these points were located in the Baltic Sea. Two histograms were drawn to visualize the distribution of distances between hares and wetlands and inland waters. One over the distances between ft+ and ft- hare coordinates and nearest wetland, and one over distances between hare coordinates and nearest inland water. Again, corresponding boxplots were drawn for better visualization of coordinate data.

2.2 Field study on *Francisella tularensis* in water

To explore presence of *F. tularensis* in water nearby sites where hares had been infected, a total of 56 water samples were collected in 14 areas. In each area, one beaver system and one lake were sampled, each with one biofilm sample and one surface water sample (depth 30-50 cm). This generated four samples per area, each consisting of 20 l of water filtered through an Asahi Kasei REXEED 25A ultra-filter. The Asahi Kasei REXEED 25A is a high flux dialyze filter with a membrane surface area of 2.5 m^2 distributed over hollow, wavy fibers, each with an internal diameter of 185 µm.

2.2.1 Selection of localities

The data from SVA on hares infected with tularemia was used to choose sampling sites. Two clusters of seven sites each were chosen: One in northern Sweden (Västerbotten-Norrbotten) and one in Central Sweden (Gästrikland-Västmanland-Dalarna). Within a buffer zone of 10 km around the 14 SVA data-points for tularemia positive hares, beaver systems were detected via aerial photos from Lantmäteriet (2020) using QGIS (QGIS Association 2021). The aerial photos (orthophoto) from 2016-2020 were systematically screened for signs of beaver activity, including presence of dying or felled trees, digging marks along the riverbanks, canals of irregular, or unnatural direction or shape, and damming of water. For each of the 14 data-points, one beaver locality and one lake were identified and chosen as water sampling sites, both within the buffer of 10 km. All localities were associated with hares testing positive for tularemia in 2019 or later, with one exception (sample 2, appendix 2) being from 2016. All sampling sites were located within the boreal and southern boreal vegetation zones (Ahti et al. 2022).

2.2.2 Field method

At each site, I took two water samples using sterile equipment. First, I put a watering can at the water surface and collected 20 l of biofilm, that was strained and filtered through a kitchen strainer, a funnel with a finer steel mesh, and lastly an ultra-fine masked nylon mesh. The second water sample was 20 l of surface water (depth 30-50 cm), handled and treated like the biofilm sample. Coordinates of each sampling site was saved using GPS. Each site was documented with camera, and general characteristics of the water and surrounding environment were put in a field protocol. The pre-filtered water was transported in two separate water drums to the filtering station. A pressure gauge (0~2 bar) with adapter fitting L/S 36 (9.5 mm) tube was installed at the top of a stand holding a fresh Asahi Kasei REXEED 25A ultra-filter, to which ~30 cm L/S 24 (6.4 mm) tube was fixated using pipe clips and a ~4 cm piece of L/S 36 tube for splicing. The

other end of the L/S 24 tube was connected to the uppermost input on the filter's red end using a luer fitting. Approximately 2 m of L/S 36 tube was connected to the pressure gauge, and the other end was put to the bottom of the filled biofilm water drum and then connected to the pump head of a Masterflex® E/S™ Portable pump (model no. 07571-00 07571-05, with recommended Masterflex® pump head installed). The pump was plugged into a charged car battery using an adapter for EU-standard plug. A draining tube was installed in the ultra-filter's outlet, perpendicular to the filter on its red end, leading filtered water into a bucket (fig. 3). Before starting the pump, the filter at question was marked with an ID referring to site and depth of sampling.

When the pump was run, the pressure was adjusted to stay around 1 bar, ± 0.1 bar, using the pump's speed control. When all 20 l had been filtered, the filter was removed and sealed using powder-free vinyl gloves and mask that were directly disposed in a biohazard waste bag for later autoclavation. The filter was then put in double plastic bags sealed with cable tie, with the outer bag containing a label with the same sample-ID as written directly on the filter.

To avoid any contamination of next coming samples; tubes, drums, watering cans, funnels and mesh were cleansed and sterilized in three steps using tap water (2 l per sample), HCl 0.2 molar (~0.3 l per sample), and finally distilled water (1 l per sample). With sterilized equipment, the procedure was repeated for the surface water, after which the equipment was again sterilized. The samples were kept in portable refrigerators at 4°C during the field work, and later transported to a cold room keeping 8 °C where they were stored until elution 15 weeks later.

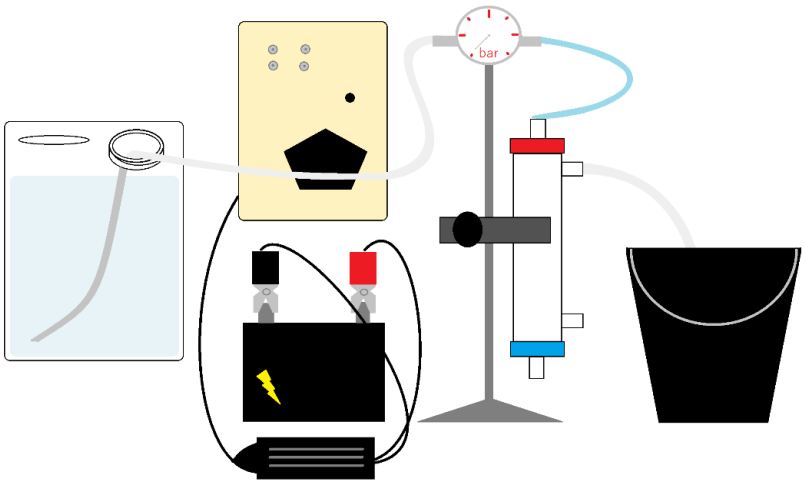


Figure 3. Schematic illustration of the setup of the filtering station. From the left: Water drum, pump, battery with adapter, ultra-filter connected to the pressure gauge, a bucket. The L/S 24 tube is drawn in blue, and the 36 tube in grey.

2.2.3 Elution of water samples in laboratory

Two stock solutions á 30 ml were mixed for the 56 water samples. The first contained 3 ml tween (100%), 0.3 ml silicon anti-foaming agent (100%), and 27.7 ml distilled water. The second stock solution contained 3 g $NaPO_3$ and 30 ml distilled water. An eluent was mixed using 1 part saline solution and 0.001 parts of each stock solution.

Outside of safety cabinet:
For each sample, one 0.5 l bottle, 3 pcs of 50 ml falcon tubes, and 6 pcs of 2 ml Eppendorf tubes were labeled with sample ID. 500 ml of eluent was poured in a 1 l bottle. These bottles and tubes, together with the filled Asahi Kasei ultra-filter at question, a sterile open cap (cap fitting 0.5 l bottle with a centered hole measuring Ø 6,4 mm, and a smaller air ventilator) and sterile L/S 24 tubes (100 cm and 40 cm) were brought into a class II safety cabinet.

Inside of safety cabinet:
The Masterflex® stationary drive (with recommended Masterflex® pump head installed) was set to 650 ml/minute, and tube size to 24. The filter was set up in a stand with a clamp, with its blue end facing up. The red, bottom end was connected to 40 cm of L/S 24 tube, using a Masterflex® luer fitting using x-small cable ties. The other end of the tube was connected to the empty 0.5 l bottle, through the open cap. The horizontal input at the blue end of the filter was connected to 100 cm L/S 24 tube, using a short (~7 cm) piece of L/S 36 tube as a splice. The tube was then put in the head of the pump before that was closed. The loose end of the tube was put in the 1 l bottle containing 0.5 l of eluate, situated on a tilted bottle holder. The installation then looked like in figure 4, below.

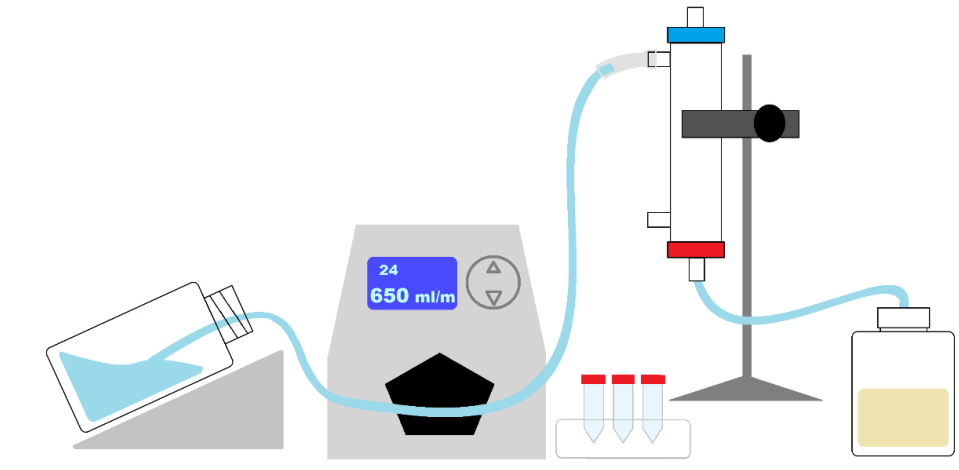


Figure 4. Setup of the Masterflex® drive inside of the safety cabinet.

The pump was started with the end of the tube held to the bottom of the bottle so that as much of the eluate as possible could go through the filter. When no more eluate was pumped into the filter, the pump was switched off. The head was then opened, and extant fluid put in a glass for wastewater. Both tubes could then be disconnected, and the filter was resealed and later autoclaved. The cap of the 0.5 l bottle containing the eluate was changed to the solid cap and then carefully turned upside down a few times to mix the sample. The eluate was then poured into 3 pcs. of labeled falcon 50 ml tubes, ~45 ml in each. The bottled sample was put for storage in cold room. Equipment was sterilized with ethanol 70% and was reused for next-coming elution.

Outside of safety cabinet:
The falcon tubes were centrifuged for 90 minutes at 3000 RPM, temperature set to 8°C.

Inside of safety cabinet:
After centrifugation, the supernatant was poured into the wastewater glass. Supernatant in samples with softer pellets that did not allow pouring, was gently removed with an Eppendorf 100-1000 µl pipette, using 1 ml filter tips. Thereafter, 1 ml of saline was added to each sample using pipette with regular 1 ml tips. Pellets were dissolved using a vortex before the sample was put into two Eppendorf tubes labeled with sample ID. In these tubes, all samples were homogenized and prepared for PCR analysis. After yet another 7 weeks, all samples were sent to the Centre for Infectious Disease Control, Netherlands for analysis.

2.2.4 Statistical analysis

When samples had been analyzed for tularemia, I constructed a data frame containing columns for x-coordinate, y-coordinate, z (elevation), sample ID, water type (beaver system or lake), sampling depth and tularemia status. I then ran a logistic regression analysis using RStudio for the coordinate variables. The categorical variables, water type and sampling depth were separately tested against tularemia status using chi-squared tests.

3. Results

3.1 Landscape analyses

The three logistic regression models all resulted in significant positive correlations between tularemia and *soil moisture*, and significant negative correlations with *spruce forest* (tables 1-3). The variable *inland water* had significant positive correlations with tularemia in the models for 1300 and 1600 m (table 2, 3). *Deciduous forest* had significant positive correlations with tularemia in the models for 500 and 1300 m (table 1, 2). *Deciduous hardwood forest* had significant negative correlations with tularemia in the models for 500 and 1600 m (table 1, 3). None of the models resulted in a significant correlation between tularemia and the wetland variable. Correlations with soil moisture for the two hare groups are visualized in conditional density plots and boxplots in figures 5 A-C and 6 A-C, respectively.

Table 1. Summaries from logistic regression for buffer zones of 500 m. P-values <0.0066 are given in bold. Min and max deviance residuals were -2.064 and 2.497, respectively. Degrees of freedom was 338 and McFadden's R^2 was 0.134.

Variable	*Estimate*	*SE*	*p-value*
Soil moist	0.031	0.008	**<0.001**
Wetland area	0.017	0.034	0.614
Inland water area	0.022	0.009	0.018
Pine forest, not on wetland	0.019	0.012	0.136
Spruce forest, not on wetland	-0.077	0.028	**0.007**
Mixed forest area, not on wetland	0.004	0.024	0.861
Deciduous forest, not on wetland	0.049	0.017	**0.004**
Deciduous hardwood forest, not on wetland	-0.089	0.030	**0.003**

Table 2. Summaries from logistic regression for buffer zones of 1300 m. P-values <0.0066 are given in bold. Min and max deviance residuals were -1.817 and 2.986, respectively. Degrees of freedom was 338 and McFadden's R^2 was 0.156.

Variable	*Estimate*	*SE*	*p-value*
Soil moist	0.032	0.010	**<0.001**
Wetland area	0.068	0.030	0.025
Inland water area	0.036	0.011	**<0.001**
Pine forest, not on wetland	0.024	0.014	0.098
Spruce forest, not on wetland	-0.076	0.027	**0.004**
Mixed forest area, not on wetland	0.025	0.029	0.389
Deciduous forest, not on wetland	0.062	0.023	**0.007**
Deciduous hardwood forest, not on wetland	-0.100	0.040	0.012

Table 3. Summaries from logistic regression for buffer zones of 1600 m. P-values <0.0066 are given in bold. Min and max deviance residuals were -1.777 and 2.971, respectively. Degrees of freedom was 338 and McFadden's R^2 was 0.160.

Variable	*Estimate*	*SE*	*p-value*
Soil moist	0.030	0.010	**0.002**
Wetland area	0.061	0.030	0.042
Inland water area	0.037	0.011	**<0.001**
Pine forest, not on wetland	0.023	0.014	0.103
Spruce forest, not on wetland	-0.090	0.029	**0.002**
Mixed forest area, not on wetland	0.049	0.032	0.126
Deciduous forest, not on wetland	0.059	0.025	0.016
Deciduous hardwood forest, not on wetland	-0.121	0.043	**0.005**

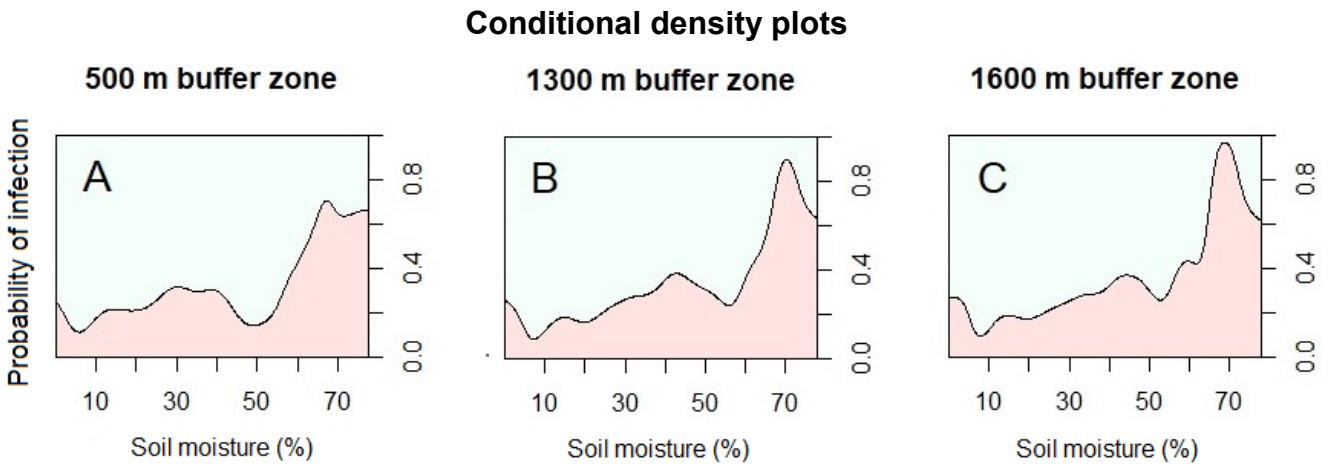


Figure 5 A-C. Conditional density plots of the change in tularemia status as a function of mean percentage of soil moisture in buffer of sizes500 m (A), 1300 m (B) and 1600 m (C). Y-axis describes the probability of a hare to have tularemia (as a function of soil moisture). Red area describes probability of tularemia, green area describes probability of not having tularemia. Maximum soil moisture was 80%.

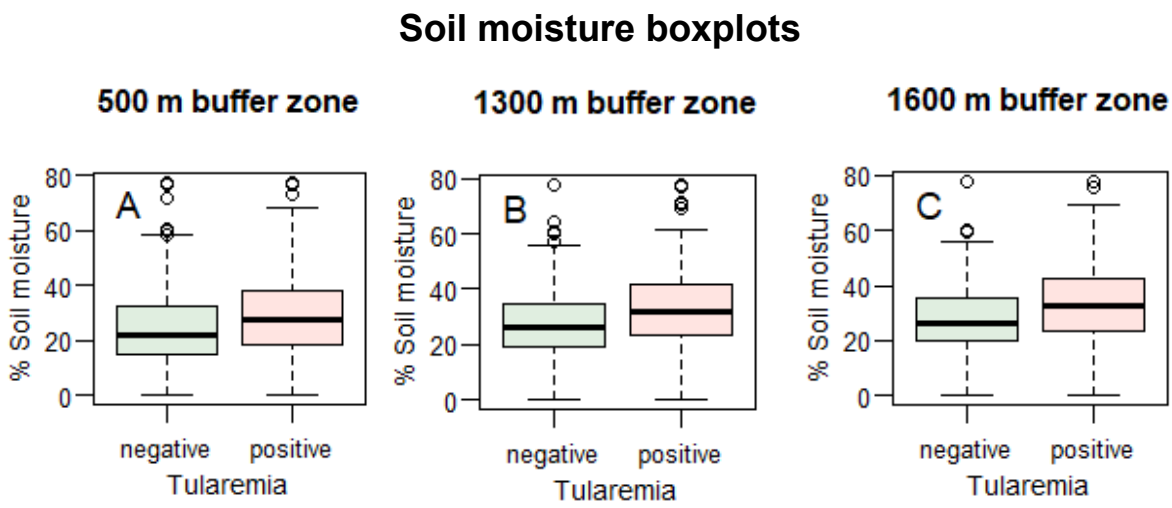


Figure 6 A-C. Boxplots showing soil moisture (%) in the three buffer zones sizes; 500 m (A), 1300 m (B), and 1600 m (C), around sites where tularemia positive and negative hares have been found deceased. Soil moisture differ significantly between the two hare groups, for all three buffer sizes. Boxes show median in bold, and upper and lower quantiles. Whiskers show data min and max, and circles show outliers

The logistic regression on wetland and inland water proximity showed no significant difference in distance to nearest wetland between tularemia positive, and tularemia negative hares (p = 0.551; table 4). Similarly, there was no significant difference in distance to nearest inland water between tularemia positive and tularemia negative hares (p = 0.178). Y-coordinates (latitudes) for the ft- buffers were significantly lower than y-coordinates of the ft+ buffers (p <0.001; table 4). X-coordinates (longitudes) had no significant effect on tularemia status in hares. Similar results were illustrated using boxplots (fig 7 A, B). Histograms over distances between hares and wetlands and inlands waters (fig. 8 A, B) have a right skewed distribution, demonstrating that most hares are in close proximity to wetlands and inland waters. Distances between hares and wetlands are generally shorter than that to lakes and watercourses.

Table 4. Summary of logistic regression model that compared coordinates and water proximity of tularemia positive, and negative hares found deceased in Sweden. Significant results are given in bold. Distance variables refer to distances between coordinates where hares have been found and nearest wetland or inland water, i.e. lake or watercourse. Min and max deviance residuals were -1.747 and 2.202, respectively. Degrees of freedom was 449 and McFadden's R^2 was 0.125.

Variable	*Estimate*	*SE*	*p-value*
Longitude (X)	-1.421e-06	1.169e-06	0.224
Latitude (Y)	3.180e-06	5.420e-07	**4.46e-09**
Wetland distance	7.492e-05	3.081e-04	0.808
Inland water distance	-1.088e-04	2.315e-04	0.638

Coordinate boxplots

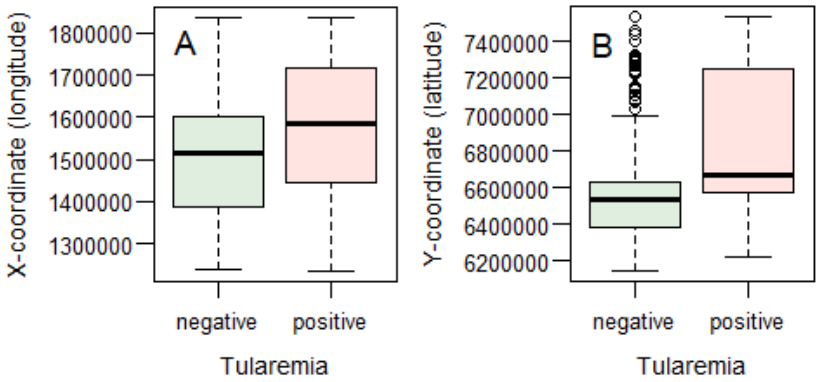


Figure 7 A, B. Boxplots showing the distribution of hares testing negative, and positive for tularemia along the x-coordinate (A) and y-coordinate (B), respectively. Y-coordinates differ significantly between the two hare groups, whilst x-coordinates do not. Coordinates run from west (low) to east (high) and from south (low) to north (high). Boxes show median in bold, and upper and lower quantiles. Whiskers show data min and max, and circles show outliers. Coordinates are for RT90 2.5 gon V projection.

Histograms over water proximity

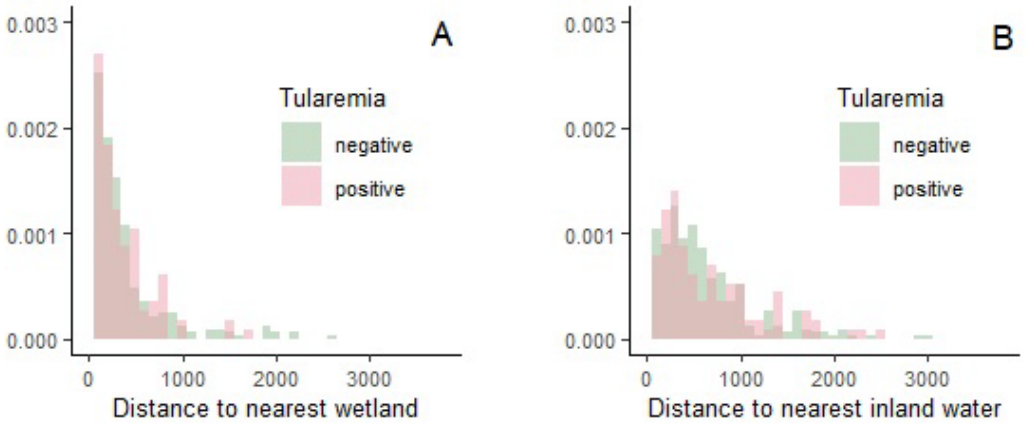


Figure 8 A, B. Histograms showing densities (y-axis) of distances (x-axis) between coordinates of deceased hares found in Sweden and nearest wetland (A), and inland water (B) with an area >100 m^2. Red bars show tularemia positive hares, green bars show tularemia negative hares. Distances are given in meters.

3.2 Water samples

Six of the 56 water samples were positive for tularemia, i.e., 11%. These were all taken from beaver wetlands, and all lake samples came back negative for tularemia. The localities testing positive showed tularemia contamination in both biofilm and surface samples. Two of the positive samples were taken in central Sweden, Dalarna and Västmanland County, and one in the north, Norrbotten County (fig. 9). All three localities were associated with hares testing positive for tularemia in 2019, and one of them was in close proximity to two hares testing positive in 2021. Locality details are provided in appendix 2.

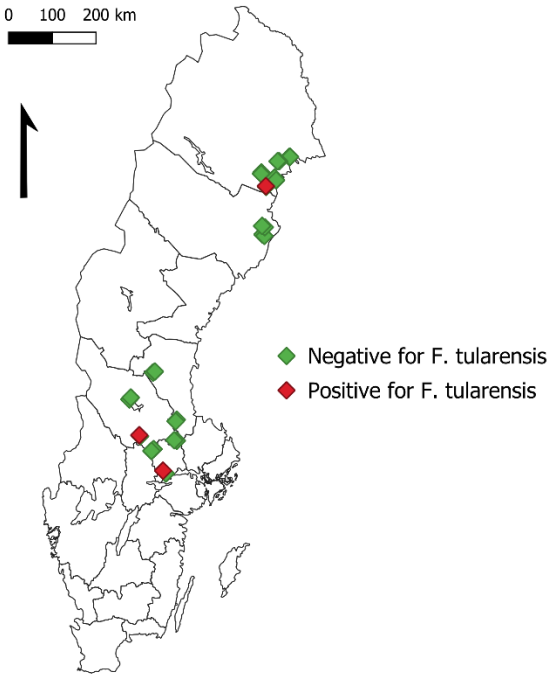


Figure 9. Map illustrating the distribution of the 28 sampling sites, distributed over 14 areas, and their tularemia status. Positive samples were taken in Dalarna, Västmanland, and Norrbotten County. Borders show Swedish county borders.

Chi-square test gave a significant correlation between tularemia status and water type, with $X^2 = 4.6667$ and $p = 0.031$. As all of the three positive localities showed contamination of *F. tularensis* in both biofilm and surface water, no chi-square test was needed to discard any impact of sampling depth. Logistic regression model showed no significant correlation between tularemia status and the variables x-coordinate, y-coordinate, or z (elevation).

4. Discussion

Landcover analyses including logistic regressions, conditional density plots and boxplots, show a clear pattern where an increased soil moisture and local water accessibility favor the occurrence of tularemia in Swedish hares. Findings concur with results from previous studies (Svensson et al. 2009; Desvars et al. 2015). Sampling of lake- and beaver wetland water at two different depths found *F. tularensis* in three beaver systems, at both sampling depths. None of the water samples taken in lakes showed contamination of *F. tularensis*.

In logistic regression models, soil moisture had significant positive effects on tularemia status for all three buffer sizes. In addition, inland water proportion in the two larger buffers had a significant, positive effect on tularemia in hares. These findings support my hypothesis that an increase in soil moisture and water availability increases the occurrence of tularemia in hares. The apparent correlation between wet environments and tularemia is most likely explained by the availability of mosquito vectors and the bacterium's enabled survival with access to water, and plausibly protozoa. Water accessibility is critical for mosquitoes' life cycle as the larval and pupal stages are aquatic (Schäfer et al. 2006; Lundström et al. 2011), and hence the waterborne cycle of tularemia. High levels of soil moisture likely correlate with occurrence of small water accumulations, essential for mosquitoes' reproduction.

Looking at the conditional density plots, the critical point in soil moisture seems to be at 50-55%, after which the curve for tularemia rockets for all three buffer sizes. An optimum soil moisture for tularemia occurrence appears to be at about 70%, where adjacent dead hares are most likely infected with tularemia. Wetter environments may allow for more permanent water bodies and thereby favor vector predation (Schäfer et al. 2006), which advocates for further investigation of findings of Schäfer et al. (2006), suggesting that permanent wetlands have a hampering effect on extensive mosquito population growth. Looking at time series from 1860-2001, it is clear that Sweden is getting warmer and wetter, especially during the summers (Alexandersson 2002). As summers of heavy precipitation combined with high temperatures favor the vectors of tularemia, future summers are likely to come with large outbreaks in both humans and hares. With global warming it is also likely that incidence of tularemia in humans will increase in the coming decades resulting from longer vector periods. In addition, as tick species associated with tularemia are expanding northward in Sweden (Jaenson et al. 2012; Gehringer et al. 2013; Maurin & Gyuranecz 2016), it is possible that their involvement in the Swedish tularemia cycle may be enhanced by global warming.

The positive correlation between the proportion of inland water and tularemia was found for buffers of 1300 m and 1600 m. This is in accordance with previous findings by Desvars et al. (2015), where the local proportion of inland

water was linked to occurrence of tularemia in humans. The missing correlation between inland water and tularemia for the 500 m buffer may indicate a need for a larger perspective for geographical analyses regarding tularemia and its hosts and vectors. Desvars et al. addressed the need for further investigations concerning water proximity in outbreaks of tularemia. In my analysis comparing wetland and inland water distances to ft- and ft+ hares, respectively, no significant difference in wetland or inland water proximity was found between the hare groups. This suggests that water abundance rather than water proximity controls the occurrence of tularemia in hares. Mosquito vectors are mobile in their adult stage searching for blood-meals, yet selective regarding habitat (Becker 2010); assuming that a greater water areal helps mosquitoes' habitat localization, it is reasonable that water area, rather than water proximity is the limiting factor for the spread of tularemia from vector to host.

Contrasting my hypothesis, an increase in wetland area within buffers had no significant effect on tularemia incidence in hares. Whether this effect would be similar regarding human incidence is beyond the scope of this study. Why wetland area lacked the same effect on tularemia as inland water might have its explanation in the biology of *F. tularensis*: The replication of *F. tularensis* is pH sensitive and suggested optimal at a pH between 5.8 and 6.3, and fully haltered at pH <4.8 (Klimentova et al. 2019). In comparison to lakes, many wetlands have a relatively low pH (Rydin et al. 2013). Low pH wetlands may thus inhibit the replication of *F. tularensis* in Swedish wetlands and hence buffer the spread of tularemia. This finding contrasts with previous suggestions of wetland restauration leading to increased activity of *F. tularensis* (Svensson et al. 2009).

A study by Shäfer et al. (2008) compared effects from wetland type on mosquito community and found no effects on mosquito abundance, but on species assemblage: This may imply an altering importance of different mosquito species on tularemia transmission, rather than solely mosquito presence. If different species of mosquitoes are of altered importance in the epizoology of tularemia, it is possible that a deeper analysis taking account for wetland types is necessary, instead of treating all wetlands as one variable. It is also possible that hares infected from other sources than vectors, such as in contact with other, infected hares, impact the results as such infections are less dependent on water and wetland accessibility. E.g., secondary consumption on cecotropes, i.e., soft hare droppings from infected hares. Bacterial transmission via cecotropes consumption has earlier been suggested for the bacterium *Mycobacterium avium*, in European brown hare (Salgado et al. 2011).

These findings do not help establish that *F. t. holarctica* is more abundant in wet environments, as epidemiological factors include more than bacterial availability. The low McFadden R^2 values indicate that the variables used are insufficient to predict tularemia outbreaks.

The variables *deciduous hardwood forest* and *deciduous forest* have

opposite correlations with tularemia status in the hares, with the former being negatively correlated and the latter positively. Deciduous forests are associated with tree species such as birch, alder, rowan, sallow and aspen (Swedish Environmental Protection Agency 2021), and species of the deciduous hardwood forests are elm, beech, ash, oak, linden tree and maple (Swedish Environmental Protection Agency 2021). The deciduous hardwood forests grow in the boreo-nemoral and nemoral zones, i.e. in the relatively warmer climate of southern Sweden (Diekmann & Sjögren 1994; Rydin & Maarel 1999) as they demand higher temperatures and relatively rich soils compared to the deciduous forest (Rydin & Maarel 1999; Portoghesi 2006). The apparent, negative correlation between deciduous hardwood forests and tularemia concurs with the claim that tularemia is less occurring in the south. This may thus be a consequence of the geographical distribution of the deciduous hardwood forests to some extent overlapping with that of lower tularemia frequencies in hares, and vice versa. The deciduous hardwood forest's demand regarding temperature is unlikely the reason for low tularemia frequencies in this region as tularemia occurs in warmer regions of Europe (Maurin & Gyuranecz 2016; European Centre for Disease Prevention and Control 2021). This matter may simply be a consequence of other factors connected to the latitudinal differences in Sweden. The negative correlation between tularemia and spruce forests on the other hand, is harder to assess. It cannot be explained by the spruces moist preferences as they thrive on mesic to very moist, rich soils (Portoghesi 2006); similar to the environments favoring *F. tularensis*. Additionally, no correlation between the soil moist and the spruce forest variables was found in the dataset. Nevertheless, spruces have a competitive advantage over other plant species in acidic soils (Rydin & Maarel 1999) and may thus be overrepresented in areas with a pH disfavoring *F. tularensis* replication. This could explain the negative correlation between the local spruce forest area and the occurrence of tularemia in hares.

The second logistic regression model shows significant differences between tularemia positive, and negative locations with regards to longitude and latitude. Traditionally, tularemia has been of little concern in southern parts of Sweden (Dahlstrand et al. 1971; Mörner & Sandstedt 1983; Tärnvik et al. 1996), and these results together with the visualization of the matter in figure 2 A, correspond to the idea that northern areas are more affected; possibly an effect of the great abundance of mosquitoes in these latitudes (Schäfer et al. 2006; Schäfer & Lundström 2009; Lundström et al. 2013). The acidified lakes found predominantly in southern Sweden (Fölster & Wilander 2002; Moldan et al. 2013) could contribute to the low frequency of tularemia cases in these areas as low pH affects *F. tularensis'* replication negatively (Klimentova et al. 2019). Acidification is mainly a consequence of fertilization and hence eutrophication, and the cultivated lands of southern Sweden are thus nutrient rich with low pH and leach to adjacent waterbodies (Nohrstedt 2001). As nutritious water boosts the

growth of *F. tularensis* (Thelaus et al. 2009) it is possible that this effect is negligible, or at least buffered from high N and/or P. However, previous studies imply an ongoing, southward expansion of tularemia cases (Rydén et al. 2009; Folkhälsomyndigheten 2021); potentially a growing issue for the red listed Swedish subspecies of mountain hare, *L. timidus sylvaticus*, having its distribution in southern Sweden.

As hypothesized, *F. tularensis* can persist in waters associated with beaver activity, and according to my results, it is far less likely to find the bacterium in lakes: This as all the samples positive for tularemia came from beaver systems. Yet, the amount of data is scarce, and more extensive sampling efforts are needed to make more rigorous estimates on the beaver's role in the cycle of *F. tularensis*. The samples in this study were taken in both stagnant water and flowing canals. The three localities where tularemia was found, had little in common. One was an extremely shallow ditch with clayey bottom, located by a field. One was a broad, seemingly stagnant canal with brown-reddish water surrounded by many felled trees, and some standing trees with beaver bite marks, located in an area of vegetated and arable land. The third, northernmost locality was a stream running through a marshy forested area. What they might have in common is however the nutritious soils due to forestry and agriculture in the areas. Nutrients help the bacteria to grow (Thelaus et al. 2009), and nutrition levels are generally higher in wetlands compared to lakes as they function as "nutrient traps" for the water passing through (Hansson et al. 2005; Verhoeven et al. 2006). Another possible contribution to this pattern is the physical activity and digging behavior of beavers, that increase water turbidity as sediment gets suspended (Hood & Larson 2014, 2015; Law et al. 2016). As *F. tularensis* is light sensitive, environments like these could to an extent protect the bacterium from destructive sun light and hence favor its survival (Friend 2006). Moreover, mud samples from beaver ponds where local beavers have been infected with tularemia, has been shown to contain live bacteria of *F. tularensis* (Jellison et al. 1942).

The three localities positive for *F. tularensis* were contaminated at both sampling depths, i.e., biofilm and ~30 cm below surface, indicating that the bacterium in both flowing and stagnant water is not restricted to the biofilm which is common for other bacteria (Costerton et al. 1987; Huws et al. 2005). For the evaluation of the method, I could not be any happier: We managed to detect *F. tularensis* in natural waters, and no apparent cross-contamination did occur, and hence the disinfection method with ethanol and HCl (0.2 molar) is approved.

Previous studies have shown correlations between tularemia and lakes (Svensson et al. 2009; Desvars et al. 2015), in accordance with what I found analyzing landcover properties. However, out of my 28 samples taken in lakes, none tested positive for tularemia. The domination of beaver system samples among tularemia positive samples may be explained by the presence of infected mammals, protozoa, and high levels of vectors allowing the bacterium to persist in

the area. In addition, these environments, if not too acidic, are excellent for both bacterial growth following high nutrient levels (Thelaus et al. 2009). As the water volume is smaller in a canal compared to the sampled lakes, it is also possible the bacterium was present but much more diluted in lakes and therefor undetectable.

Why my field study found a correlation between wetlands and tularemia, that the landcover analysis missed could partially be explained by the fact that the water in beaver systems is determined as lake or watercourse in the landcover data, and hence a part of the inland water variable. Thus, these beaver systems may in fact contribute to the significant correlation found between tularemia in hares and inland water. How beaver activity impacts wetland pH has been studied with various results: Evidence of both elevation, decrease and no significant changes in pH related to beaver activity has been observed (Adams et al. 1995; Margolis et al. 2001; Little et al. 2012). For future studies, testing of water pH, turbidity and levels of N and P could contribute to a deeper understanding of the processes controlling the occurrence of *F. tularensis* in natural waters. In addition, it would be valuable to obtain a more detailed dataset on wetland properties for future analyses, allowing for comparisons between different wetland types, e.g., in terms of beaver activity and water biogeochemistry.

5. Conclusion

My results from Sweden show how factors as soil moisture and wet environments favor the occurrence of tularemia and its causative agent, the bacterium Francisella tularensis. In addition, I exclusively found *F. tularensis* in water samples from beaver wetlands, but landcover analysis showed an enhanced risk of tularemia in areas with a great inland water area: Proportion of water appears to have a stronger effect on tularemia incidence in hares, compared to proximity to water. Findings may imply concern regarding wetland restauration, as increased soil moisture might cause higher probability of tularemia infection in wildlife.

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References

Abd, H., Johansson, T., Golovliov, I., Sandström, G. & Forsman, M. (2003). Survival and Growth of *Francisella tularensis* in *Acanthamoeba castellanii*. *Applied and Environmental Microbiology*, 69 (1), 600–606. https://doi.org/10.1128/AEM.69.1.600-606.2003

Adams, P.W., University of Waterloo, & Department of Biology (1995). *The effects of beaver (castor canadensis) on boreal forest peatlands and keys to the sphagnum of Ontario*. [University of Waterloo].

Ahangari Cohan, H., Jamshidian, M., Rohani, M., Moravedji, M. & Mostafavi, E. (2020). Surveillance of Francisella tularensis in surface water of Kurdistan province, west of Iran. *Comparative Immunology, Microbiology and Infectious Diseases*, 69, 101419. https://doi.org/10.1016/j.cimid.2020.101419

Ahti, T., Hamet-Ahti, L. & Jalas, J. (2022). Vegetation zones and their sections in northwestern Europe. 44

Alexandersson, H. (2002). *Temperature and precipitation in Sweden 1860-2001*. (104). Norrköping: SMHI - Meteorologi.

Andrén, H. & Liberg, O. (2008). *Slutrapport - Lodjursprojektet*. (Scandlynx). Riddarhyttan: Grimsö forskningsstation, Institutionen för ekologi, SLU.

Balci, E., Borlu, A., Kilic, A.U., Demiraslan, H., Oksuzkaya, A. & Doganay, M. (2014). Tularemia outbreaks in Kayseri, Turkey: An evaluation of the effect of climate change and climate variability on tularemia outbreaks. *Journal of Infection and Public Health*, 7 (2), 125–132. https://doi.org/10.1016/j.jiph.2013.09.002

Becker, N. (red.) (2010). *Mosquitoes and their control*. 2nd ed. Heidelberg: Springer.

Berdal, B.P. (1996). Field investigations of tularemia in Norway. 5

Brantsaeter, A.B., Krogh, T., Radtke, A. & Nygård, K. (2007). Tularaemia outbreak in northern Norway. *Weekly releases (1997–2007)*, 12 (13). https://doi.org/10.2807/esw.12.13.03165-en

Broman, T., Thelaus, J., Andersson, A.-C., Bäckman, S., Wikström, P., Larsson, E., Granberg, M., Karlsson, L., Bäck, E., Eliasson, H., Mattsson, R., Sjöstedt, A. & Forsman, M. (2011). Molecular Detection of Persistent *Francisella tularensis* Subspecies *holarctica* in Natural Waters. *International Journal of Microbiology*, 2011, 1–10. https://doi.org/10.1155/2011/851946

Carvalho, C.L., Lopes de Carvalho, I., Zé-Zé, L., Núncio, M.S. & Duarte, E.L. (2014). Tularaemia: A challenging zoonosis. *Comparative Immunology, Microbiology and Infectious Diseases*, 37 (2), 85–96. https://doi.org/10.1016/j.cimid.2014.01.002

Černý, Z. (2001). *Changes of the epidemiology and the clinical picture of tularemia in Southern Moravia (the Czech Republic) during the period 1936–1999*. (European Journal of Epidemiology, 17: 637–642). Brno – Bohunice, Jihlavska´ 20, Czech Republic: Clinic of Infectious Diseases (Faculty Hospital).

Chandrasegaran, K., Lahondère, C., Escobar, L.E. & Vinauger, C. (2020). Linking Mosquito Ecology, Traits, Behavior, and Disease Transmission. *Trends in Parasitology*, 36 (4), 393–403. https://doi.org/10.1016/j.pt.2020.02.001

Constable, P.D., Hinchcliff, K.W., Done, S.H. & Grünberg, W. (red.) (2017). Systemic and Multi-Organ Diseases. *Veterinary Medicine (Eleventh Edition)*. W.B. Saunders, 2002–2214. https://doi.org/10.1016/B978-0-7020-5246-0.00021-8

Costerton, J.W., Cheng, K.J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M. & Marrie, T.J. (1987). Bacterial biofilms in nature and disease. *Ann. Rev. Microbiol.*, 41 :435-64

Cunningham, J.M., Calhoun, A.J.K. & Glanz, W.E. (2006). Patterns of Beaver Colonization and Wetland Change in Acadia National Park. *Northeastern Naturalist*, 13 (4), 583–596. https://doi.org/10.1656/1092-6194(2006)13[583:POBCAW]2.0.CO;2

Dahl, F. (2005). Distinct seasonal habitat selection by annually sedentary mountain hares (Lepus timidus) in the boreal forest of Sweden. *European Journal of Wildlife Research*, 51 (3), 163–169. https://doi.org/10.1007/s10344-005-0095-y

Dahlstrand, S., Ringertz, O. & Zetterberg, B. (1971). Airborne Tularemia in Sweden. *Scandinavian Journal of Infectious Diseases*, 3 (1), 7–16. https://doi.org/10.3109/inf.1971.3.issue-1.02

Decors, A., Lesage, C., Jourdain, E., Giraud, P., Houbron, P., Vanhem, P. & Madani, N. (2011). Outbreak of tularaemia in brown hares (Lepus europaeus) in France, January to March 2011. *Eurosurveillance*, 16 (28). https://doi.org/10.2807/ese.16.28.19913-en

Delaney, M.A., Treuting, P.M. & Rothenburger, J.L. (2018). Lagomorpha. *Pathology of Wildlife and Zoo Animals*. Elsevier, 481–498. https://doi.org/10.1016/B978-0-12-805306-5.00019-5

Desvars, A., Furberg, M., Hjertqvist, M., Vidman, L., Sjöstedt, A., Rydén, P. & Johansson, A. (2015). Epidemiology and Ecology of Tularemia in Sweden, 1984–2012. *Emerging Infectious Diseases*, 21 (1), 32–39. https://doi.org/10.3201/eid2101.140916

Diekmann, M. & Sjögren, E. (red.) (1994). *Deciduous forest vegetation in Boreo-nemoral Scandinavia*. Uppsala: Svenska Växtgeografiska Sällskapet. http://www.diva-portal.org/smash/get/diva2:565456/FULLTEXT01.pdf

Ecke, F., Levanoni, O., Audet, J., Carlson, P., Eklöf, K., Hartman, G., McKie, B., Ledesma, J., Segersten, J., Truchy, A. & Futter, M. (2017). Meta-analysis of environmental effects of beaver in

relation to artificial dams. *Environmental Research Letters*, 12 (11), 113002. https://doi.org/10.1088/1748-9326/aa8979

Eiseltová, M. (red.) (2010). *Restoration of Lakes, Streams, Floodplains, and Bogs in Europe: Principles and Case Studies*. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-90-481-9265-6

Ekdahl, K. & Twisselmann, B. (2001). Epidemics of tularaemia in Sweden and Finland. *Weekly releases (1997–2007)*, 5 (2). https://doi.org/10.2807/esw.05.02.01825-en

Eliasson, H., Lindbäck, J., Nuorti, J.P., Arneborn, M., Giesecke, J. & Tegnell, A. (2002). The 2000 Tularemia Outbreak: A Case-Control Study of Risk Factors in Disease-Endemic and Emergent Areas, Sweden. *Emerging Infectious Diseases*, 8 (9), 956–960. https://doi.org/10.3201/eid0809.020051

European Centre for Disease Prevention and Control (2011a). *Annual epidemiological report 2011 : reporting on 2009 surveillance data and 2010 epidemic intelligence data*. LU: Publications Office. https://data.europa.eu/doi/10.2900/14803 [2021-09-15]

European Centre for Disease Prevention and Control (2011b). *Annual epidemiological report 2011 : reporting on 2009 surveillance data and 2010 epidemic intelligence data*. LU: Publications Office. https://data.europa.eu/doi/10.2900/14803 [2021-09-15]

European Centre for Disease Prevention and Control (2021). *Tularaemia. In: ECDC. Annual epidemiological report for 2019*. Stockholm.

European Environment Information and Observation Network (2018). *Article 17 web tool*. https://nature-art17.eionet.europa.eu/article17/species/report/?period=5&group=Mammals&country=SE&region=CON

Folkhälsomyndigheten (2021). *Sjukdomsstatistik: Harpest.* https://www.folkhalsomyndigheten.se/folkhalsorapportering-statistik/statistik-a-o/sjukdomsstatistik/harpest/#statistics-nav

Forsman, M., Henningson, E.W., Larsson, E., Johansson, T. & SandstrÃ¶m, G. (2000). Francisella tularensis does not manifest virulence in viable but non-culturable state. *FEMS Microbiology Ecology*, 31 (3), 217–224. https://doi.org/10.1111/j.1574-6941.2000.tb00686.x

Friend, M. (2006). *Tularemia*. Reston, Va: U.S. Dept. of the Interior, U.S. Geological Survey. (Circular; 1297)

Fölster, J. & Wilander, A. (2002). Recovery from acidification in Swedish forest streams. *Environmental Pollution*, 117 (3), 379–389. https://doi.org/10.1016/S0269-7491(01)00201-9

Gehringer, H., Schacht, E., Maylaender, N., Zeman, E., Kaysser, P., Oehme, R., Pluta, S. & Splettstoesser, W.D. (2013). Presence of an emerging subclone of Francisella tularensis holarctica in Ixodes ricinus ticks from south-western Germany. *Ticks and Tick-borne Diseases*, 4 (1–2), 93–100. https://doi.org/10.1016/j.ttbdis.2012.09.001

Gren, I.-M. (1995). Costs and benefits of restoring wetlands: two Swedish case studies. *Ecological Engineering*, 4 (2), 153–162. https://doi.org/10.1016/0925-8574(94)00043-5

Gyuranecz, M. (2012). *Infectious diseases of wild mammals and birds in Europe*. (Gavier-Widén, D., Meredith, A., & Duff, J. P., red.) Hoboken: Wiley.

https://search.ebscohost.com/login.aspx?direct=true&scope=site&db=nlebk&db=nlabk&AN=466729 [2021-09-20]

Gyuranecz, M. (2014). Bacteria: Francisella tularensis. *Encyclopedia of Food Safety*. Elsevier, 442–445. https://doi.org/10.1016/B978-0-12-378612-8.00099-8

Gyuranecz, M., Rigó, K., Dán, Á., Földvári, G., Makrai, L., Dénes, B., Fodor, L., Majoros, G., Tirják, L. & Erdélyi, K. (2011). Investigation of the Ecology of *Francisella tularensis* During an Inter-Epizootic Period. *Vector-Borne and Zoonotic Diseases*, 11 (8), 1031–1035. https://doi.org/10.1089/vbz.2010.0091

Gyuranecz, M., Szeredi, L., Makrai, L., Fodor, L., Mészáros, Á.R., Szépe, B., Füleki, M. & Erdélyi, K. (2010). Tularemia of European Brown Hare (*Lepus europaeus*): A Pathological, Histopathological, and Immunohistochemical Study. *Veterinary Pathology*, 47 (5), 958–963. https://doi.org/10.1177/0300985810369902

Hansson, L.-A., Bronmark, C., Anders Nilsson, P. & Abjornsson, K. (2005). Conflicting demands on wetland ecosystem services: nutrient retention, biodiversity or both? *Freshwater Biology*, 50 (4), 705–714. https://doi.org/10.1111/j.1365-2427.2005.01352.x

Hartman, G. (1994). Long-Term Population Development of a Reintroduced Beaver (Castor fiber) Population in Sweden. *Conservation Biology*, 8 (3), 713–717. https://doi.org/10.1046/j.1523-1739.1994.08030713.x

Hartman, G. (1995). Patterns of spread of a reintroduced beaver *Castor fiber* population in Sweden. *Wildlife Biology*, 1 (1), 97–103. https://doi.org/10.2981/wlb.1995.0015

Hestvik, G., Uhlhorn, H., Södersten, F., Åkerström, S., Karlsson, E., Westergren, E. & Gavier-Widén, D. (2017). Tularaemia in European Brown Hares (Lepus europaeus) and Mountain Hares (Lepus timidus) Characterized by Histopathology and Immunohistochemistry: Organ Lesions and Suggestions of Routes of Infection and Shedding. *Journal of Comparative Pathology*, 157 (2–3), 103–114. https://doi.org/10.1016/j.jcpa.2017.06.003

Hestvik, G., Warns-Petit, E., Smith, L.A., Fox, N.J., Uhlhorn, H., Artois, M., Hannant, D., Hutchings, M.R., Mattsson, R., Yon, L. & Gavier-Widen, D. (2015). The status of tularemia in Europe in a one-health context: a review. *Epidemiology and Infection*, 143 (10), 2137–2160. https://doi.org/10.1017/S0950268814002398

Hood, G.A. & Larson, D.G. (2014). Beaver-Created Habitat Heterogeneity Influences Aquatic Invertebrate Assemblages in Boreal Canada. *Wetlands*, 34 (1), 19–29. https://doi.org/10.1007/s13157-013-0476-z

Hood, G.A. & Larson, D.G. (2015). Ecological engineering and aquatic connectivity: a new perspective from beaver-modified wetlands. *Freshwater Biology*, 60 (1), 198–208. https://doi.org/10.1111/fwb.12487

Huws, S.A., McBain, A.J. & Gilbert, P. (2005). Protozoan grazing and its impact upon population dynamics in biofilm communities. *Journal of Applied Microbiology*, 98 (1), 238–244. https://doi.org/10.1111/j.1365-2672.2004.02449.x

Hörnfeldt, B., Löfgren, O. & Carlsson, B.-G. (1986). Cycles in voles and small game in relation to variations in plant production indices in Northern Sweden. *Oecologia*, 68 (4), 496–502. https://doi.org/10.1007/BF00378761

Jaenson, T.G., Jaenson, D.G., Eisen, L., Petersson, E. & Lindgren, E. (2012). Changes in the geographical distribution and abundance of the tick Ixodes ricinus during the past 30 years in Sweden. *Parasites & Vectors*, 5 (1), 8. https://doi.org/10.1186/1756-3305-5-8

Jansson, G. & Pehrson, Å. (2005). *Fältharen – långt ifrån bara på fälten*. (Fakta skog, 3). SLU - Faculty of Conservation biology. https://www.slu.se/globalassets/ew/ew-centrala/forskn/popvet-dok/faktaskog/faktaskog05/fs05-03.pdf

Jansson, G. & Pehrson, Å. (2007). The recent expansion of the brown hare (Lepus europaeus) in Sweden with possible implications to the mountain hare (L. timidus). *European Journal of Wildlife Research*, 53 (2), 125–130. https://doi.org/10.1007/s10344-007-0086-2

Jellison, W.L., Kohls, G.M., Butler, W.J. & Weaver, J.A. (1942). Epizootic tularemia in the beaver, Castor canadensis, and the contamination of stream water with Pasteurella tularensis. *American Journal of Epidemiology*, 36 (2), 168–182. https://doi.org/10.1093/oxfordjournals.aje.a118815

Kevin, M., Girault, G., Caspar, Y., Cherfa, M.A., Mendy, C., Maurin, M., Ponsart, C. & Madani, N. (2021). High-resolution melting PCR assay as a powerful tool for the epidemiological surveillance of tularemia in Western Europe. *Infection, Genetics and Evolution*, 90, 104741. https://doi.org/10.1016/j.meegid.2021.104741

Klimentova, J., Pavkova, I., Horcickova, L., Bavlovic, J., Kofronova, O., Benada, O. & Stulik, J. (2019). Francisella tularensis subsp. holarctica Releases Differentially Loaded Outer Membrane Vesicles Under Various Stress Conditions. *Frontiers in Microbiology*, 10, 2304. https://doi.org/10.3389/fmicb.2019.02304

Kugeler, K.J., Mead, P.S., Janusz, A.M., Staples, J.E., Kubota, K.A., Chalcraft, L.G. & Petersen, J.M. (2009). Molecular Epidemiology of *Francisella tularensis* in the United States. *Clinical Infectious Diseases*, 48 (7), 863–870. https://doi.org/10.1086/597261

Lantmäteriet (2020). Orthophoto View Service Digital, . https://www.lantmateriet.se/en/maps-and-geographic-information/geodataprodukter/produktlista/orthophoto-viewing-service/

Law, A., Gaywood, M.J., Jones, K.C., Ramsay, P. & Willby, N.J. (2017). Using ecosystem engineers as tools in habitat restoration and rewilding: beaver and wetlands. *Science of The Total Environment*, 605–606, 1021–1030. https://doi.org/10.1016/j.scitotenv.2017.06.173

Law, A., McLean, F. & Willby, N.J. (2016). Habitat engineering by beaver benefits aquatic biodiversity and ecosystem processes in agricultural streams. *Freshwater Biology*, 61 (4), 486–499. https://doi.org/10.1111/fwb.12721

Lindgren, E. & Jaenson, T.G.T. (2006). Fästing- och myggöverförda infektionssjukdomar i ett kommande, varmare klimat i Sverige. 10

Little, A.M., Guntenspergen, G.R. & Allen, T.F.H. (2012). Wetland vegetation dynamics in response to beaver (*Castor canadensis*) activity at multiple scales. *Écoscience*, 19 (3), 246–257. https://doi.org/10.2980/19-3-3498

Lundström, J.O., Andersson, A.-C., Bäckman, S., Schäfer, M.L., Forsman, M. & Thelaus, J. (2011). Transstadial Transmission of *Francisella tularensis holarctica* in Mosquitoes, Sweden. *Emerging Infectious Diseases*, 17 (5), 794–799. https://doi.org/10.3201/eid1705.100426

Lundström, J.O., Schäfer, M.L., Hesson, J.C., Blomgren, E., Lindström, A., Wahlqvist, P., Halling, A., Hagelin, A., Ahlm, C., Evander, M., Broman, T., Forsman, M. & Vinnersten, T.Z.P. (2013). The geographic distribution of mosquito species in Sweden. *Journal of the European Mosquito Control Association*, 31, 15

Ma, Y., Vigouroux, G., Kalantari, Z., Goldenberg, R. & Destouni, G. (2020). Implications of Projected Hydroclimatic Change for Tularemia Outbreaks in High-Risk Areas across Sweden. *International Journal of Environmental Research and Public Health*, 17 (18), 6786. https://doi.org/10.3390/ijerph17186786

Margolis, B.E., Castro, M.S. & Raesly, R.L. (2001). The impact of beaver impoundments on the water chemistry of two Appalachian streams. 58, 13

Maurin, M. & Gyuranecz, M. (2016). Tularaemia: clinical aspects in Europe. *The Lancet Infectious Diseases*, 16 (1), 113–124. https://doi.org/10.1016/S1473-3099(15)00355-2

Moinet, M., Decors, A., Mendy, C., Faure, E., Durand, B. & Madani, N. (2016). Spatio-temporal dynamics of tularemia in French wildlife: 2002–2013. *Preventive Veterinary Medicine*, 130, 33–40. https://doi.org/10.1016/j.prevetmed.2016.05.015

Moldan, F., Cosby, B.J. & Wright, R.F. (2013). Modeling Past and Future Acidification of Swedish Lakes. *AMBIO*, 42 (5), 577–586. https://doi.org/10.1007/s13280-012-0360-8

Mörner, T. (1992). The ecology of tularaemia. 11 (4), 1123–1130. https://doi.org/10.20506/rst.11.4.657

Mörner, T. & Addison, E. (2001). Tularemia. I: Williams, E.S. & Barker, I.K. (red.) *Infectious Diseases of Wild Mammals*. Ames, Iowa, USA: Iowa State University Press, 303–312. https://doi.org/10.1002/9780470344880.ch18

Mörner, T. & Sandstedt, K. (1983). A serological survey of antibodies against Francisella tularensis in some Swedish mammals. *Nordisk Veterinaermedicin*, 35 (2), 82–85

Mörner, T., Sandström, G. & Mattsson, R. (1988a). COMPARISON OF SERUM AND LUNG EXTRACTS FOR SURVEYS OF WILD ANIMALS FOR ANTIBODIES TO FRANCISELLA TULARENSIS BIOVAR PALAEARCTICA. *Journal of Wildlife Diseases*, 24 (1), 10–14. https://doi.org/10.7589/0090-3558-24.1.10

Mörner, T., Sandström, G., Mattsson, R. & Nilsson, P.-O. (1988b). INFECTIONS WITH FRANCISELLA TULARENSIS BIOVAR PALAEARCTICA IN HARES (LEPUS TUMIDUS, LEPUS EUROPAEUS) FROM SWEDEN. *Journal of Wildlife Diseases*, 24 (3), 422–433. https://doi.org/10.7589/0090-3558-24.3.422

National Veterinary Institute - SVA (2020). *Surveillance of Infectious Diseases in Animals and Humans in Sweden 2020 - Tularaemia*. (SVA:s rapportserie 68 1654-7098, 68 1654-7098). Uppsala, Sweden. https://www.sva.se/media/8d92f24de8912ec/tularaemia.pdf [2021-11-01]

Nohrstedt, H.-Ö. (2001). Response of Coniferous Forest Ecosystems on Mineral Soils to Nutrient Additions: A Review of Swedish Experiences. *Scandinavian Journal of Forest Research*, 16 (6), 555–573. https://doi.org/10.1080/02827580152699385

Odden, J., Linnell, J.D.C. & Andersen, R. (2006). Diet of Eurasian lynx, Lynx lynx, in the boreal forest of southeastern Norway: the relative importance of livestock and hares at low roe deer density. *European Journal of Wildlife Research*, 52 (4), 237–244. https://doi.org/10.1007/s10344-006-0052-4

Pavliska, P.L., Riegert, J., Grill, S. & Šálek, M. (2018). The effect of landscape heterogeneity on population density and habitat preferences of the European hare (Lepus europaeus) in contrasting farmlands. *Mammalian Biology*, 88, 8–15. https://doi.org/10.1016/j.mambio.2017.11.003

Payne, N.F. (1989). Popualtion dynamics and harvest response of beaver. *Fourth Eastern Wildlife Damage Control Conference*, (33). https://digitalcommons.unl.edu/ewdcc4/33

Pedrini, P. & Sergio, F. (2002). Regional conservation priorities for a large predator: golden eagles (Aquila chrysaetos) in the Alpine range. *Biological Conservation*, 103 (2), 163–172. https://doi.org/10.1016/S0006-3207(01)00116-1

Petrosyan, V.G., Golubkov, V.V., Zavyalov, N.A., Khlyap, L.A., Dergunova, N.N. & Osipov, F.A. (2019). Modelling of competitive interactions between native Eurasian (Castor fiber) and alien North American (Castor canadensis) beavers based on long-term monitoring data (1934–2015). *Ecological Modelling*, 409, 108763. https://doi.org/10.1016/j.ecolmodel.2019.108763

Portoghesi, L. (2006). European Forest Types. Categories and types for sustainable forest management reporting and policy. *Forest@ - Rivista di Selvicoltura ed Ecologia Forestale*, 3 (4), 462–463. https://doi.org/10.3832/efor0425-003

QGIS Association (2021). *QGIS.org*. QGIS Association. http://www.qgis.org

Rohr, J.R., Dobson, A.P., Johnson, P.T.J., Kilpatrick, A.M., Paull, S.H., Raffel, T.R., Ruiz-Moreno, D. & Thomas, M.B. (2011). Frontiers in climate change–disease research. *Trends in Ecology & Evolution*, 26 (6), 270–277. https://doi.org/10.1016/j.tree.2011.03.002

Rossow, H., Forbes, K.M., Tarkka, E., Kinnunen, P.M., Hemmilä, H., Huitu, O., Nikkari, S., Henttonen, H., Kipar, A. & Vapalahti, O. (2014a). Experimental Infection of Voles with Francisella tularensis Indicates Their Amplification Role in Tularemia Outbreaks. (Dumler, J. S., red.) *PLoS ONE*, 9 (10), e108864. https://doi.org/10.1371/journal.pone.0108864

Rossow, H., Ollgren, J., Klemets, P., Pietarinen, I., Saikku, J., Pekkanen, E., Nikkari, S., Syrjälä, H., Kuusi, M. & Nuorti, J.P. (2014b). Risk factors for pneumonic and ulceroglandular tularaemia in Finland: a population-based case-control study. *Epidemiology and Infection*, 142 (10), 2207–2216. https://doi.org/10.1017/S0950268813002999

RStudio Team (2021). *RStudio: Integrated Development Environment for R*. Boston, MA. http://www.rstudio.com/

Rubin, L.G. (2018). Francisella tularensis (Tularemia). *Principles and Practice of Pediatric Infectious Diseases*. Elsevier, 923-925.e1. https://doi.org/10.1016/B978-0-323-40181-4.00171-7

Rydén, P., Björk, R., Schäfer, M.L., Lundström, J.O., Petersén, B., Lindblom, A., Forsman, M., Sjöstedt, A. & Johansson, A. (2012). Outbreaks of Tularemia in a Boreal Forest Region Depends on Mosquito Prevalence. *The Journal of Infectious Diseases*, 205 (2), 297–304. https://doi.org/10.1093/infdis/jir732

Rydén, P., Sjöstedt, A. & Johansson, A. (2009). Effects of climate change on tularaemia disease activity in Sweden. *Global Health Action*, 2 (1), 2063. https://doi.org/10.3402/gha.v2i0.2063

Rydin, H., Jeglum, J.K. & Bennett, K.D. (2013). *The biology of peatlands*. 2nd ed. Oxford: Oxford University Press. (Biology of habitats)

Rydin, H. & Maarel, E. van der (red.) (1999). *Swedish plant geography: dedicated to Eddy van der Maarel on his 65th birthday*. Uppsala: Svenska Växtgeografiska Sällskapet. (Acta phytogeographica Suecica; 84)

Salgado, M., Manning, E.J.B., Monti, G., Bólske, G., Soderlund, R., Ruiz, M., Paredes, E., Leiva, S., Van Kruningen, H. & Kruze, J. (2011). European Hares in Chile: A Different Lagomorph Reservoir

for Mycobacterium avium subsp. paratuberculosis? *Journal of Wildlife Diseases*, 47 (3), 734–738. https://doi.org/10.7589/0090-3558-47.3.734

Schneider, M. & Sahlén, E. (2018). *Rovdjur i norra Sverige Förutsättningar, förekomst och förvaltning*. Umeå: Länsstyrelsen Västerbotten. https://www.lansstyrelsen.se/download/18.4e0415ee166afb593241fe77/1542790537498/Rovdjur%20i%20norra%20Sverige.pdf

Schulze, C., Heuner, K., Myrtennäs, K., Karlsson, E., Jacob, D., Kutzer, P., GROßE, K., Forsman, M. & Grunow, R. (2016). High and novel genetic diversity of *Francisella tularensis* in Germany and indication of environmental persistence. *Epidemiology and Infection*, 144 (14), 3025–3036. https://doi.org/10.1017/S0950268816001175

Schäfer, M.L., Lundkvist, E., Landin, J., Persson, T.Z. & Lundström, J.O. (2006). Influence of landscape structure on mosquitoes (Diptera: Culicidae) and dytiscids (Coleoptera: Dytiscidae) at five spatial scales in Swedish wetlands. *Wetlands*, 26 (1), 57–68. https://doi.org/10.1672/0277-5212(2006)26[57:IOLSOM]2.0.CO;2

Schäfer, M.L. & Lundström, J.O. (2009). The present distribution and predicted geographic expansion of the floodwater mosquito Aedes sticticus in Sweden. *Journal of Vector Ecology*, 34 (1), 141–147. https://doi.org/10.1111/j.1948-7134.2009.00017.x

Sjöberg, G., Ulevičius, A. & Belova, O. (2019). *Good practices for management of beavers and beaver ponds in the Baltic Sea Region*

Sjöstedt, A. (2007). Tularemia: History, Epidemiology, Pathogen Physiology, and Clinical Manifestations. *Annals of the New York Academy of Sciences*, 1105 (1), 1–29. https://doi.org/10.1196/annals.1409.009

Stewart, S.J. (1996). Tularemia: Association with hunting and farming. *FEMS Immunology and Medical Microbiology*, 13 (1996) I97-199, 3

Strand, O., Linnell, J.D.C., Krogstad, S. & Landa, A. (1999). Dietary and Reproductive Responses of Arctic Foxes to Changes in Small Rodent Abundance. *ARCTIC*, 52 (3), 272–278. https://doi.org/10.14430/arctic932

SVA (2021). Karta över harpest. Statens Veterinärmedicinska Anstalt (National Veterinary Institute). https://www.sva.se/amnesomraden/smittlage/karta-over-harpest/

Svensson, K., Bäck, E., Eliasson, H., Berglund, L., Granberg, M., Karlsson, L., Larsson, P., Forsman, M. & Johansson, A. (2009). Landscape Epidemiology of Tularemia Outbreaks in Sweden. *Emerging Infectious Diseases*, 15 (12), 1937–1947. https://doi.org/10.3201/eid1512.090487

Swedish Environmental Protection Agency (2021). National Land Cover Database Digital, Stockholm, Sweden: Naturvårdsverket. https://metadatakatalogen.naturvardsverket.se/metadatakatalogen/GetMetaDataById?id=8853721d-a466-4c01-afcc-9eae57b17b39

Thelaus, J., Andersson, A., Broman, T., Bäckman, S., Granberg, M., Karlsson, L., Kuoppa, K., Larsson, E., Lundmark, E., Lundström, J.O., Mathisen, P., Näslund, J., Schäfer, M., Wahab, T. & Forsman, M. (2014). Francisella tularensis Subspecies holarctica Occurs in Swedish Mosquitoes, Persists Through the Developmental Stages of Laboratory-Infected Mosquitoes and Is Transmissible During Blood Feeding. *Microbial Ecology*, 67 (1), 96–107. https://doi.org/10.1007/s00248-013-0285-1

Thelaus, J., Andersson, A., Mathisen, P., Forslund, A.-L., Noppa, L. & Forsman, M. (2009). Influence of nutrient status and grazing pressure on the fate of Francisella tularensis in lake water: Role of nutrient and protozoa for the fate of F. tularensis. *FEMS Microbiology Ecology*, 67 (1), 69–80. https://doi.org/10.1111/j.1574-6941.2008.00612.x

Thulin, C.-G., Winiger, A., Tallian, A.G. & Kindberg, J. (2021). Hunting harvest data in Sweden indicate precipitous decline in the native mountain hare subspecies Lepus timidus sylvaticus (heath hare). *Journal for Nature Conservation*, 126069. https://doi.org/10.1016/j.jnc.2021.126069

Tveite, H. (2022). *NNJoin*. Version: 3.1.3. http://arken.nmbu.no/~havatv/gis/qgisplugins/NNJoin

Tärnvik, A., Priebe, H. & Grunow, R. (2004). Tularaemia in Europe: An Epidemiological Overview. *Scandinavian Journal of Infectious Diseases*, 36 (5), 350–355. https://doi.org/10.1080/00365540410020442

Tärnvik, A., Sandström, G. & Sjöstedt, A. (1996). *Epidemiological analysis of tularemia in Sweden 1931-1993*. (FEMS Immunology and Medical Microbiology, 13 (1996) 20 I-104). Department of Infectious Diseases, Umeå University.

Tärnvik, A. & World Health Organization (red.) (2007). *WHO guidelines on tularaemia: epidemic and pandemic alert and response*. Geneva: World Health Organization.

University of Illinois (2016). Logistic Regression. Atlas - Department of Statistics. http://courses.atlas.illinois.edu/fall2016/STAT/STAT200/RProgramming/LogisticRegression.html

Verhoeven, J., Arheimer, B., Yin, C. & Hefting, M. (2006). Regional and global concerns over wetlands and water quality. *Trends in Ecology & Evolution*, 21 (2), 96–103. https://doi.org/10.1016/j.tree.2005.11.015

Willby, N.J., Law, A., Levanoni, O., Foster, G. & Ecke, F. (2018). Rewilding wetlands: beaver as agents of within-habitat heterogeneity and the responses of contrasting biota. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373 (1761), 20170444. https://doi.org/10.1098/rstb.2017.0444

Wilson, D.E., Mittermeier, R.A. & Cavallini, P. (red.) (2017). *Handbook of the mammals of the world - Volume 6. Lagomorphs and Rodents I*. Barcelona: Lynx Edicions : Conservation International : IUCN.

Yapar, D., Erenler, A.K., Terzi, Ö., Akdoğan, Ö., Ece, Y. & Baykam, N. (2016). Predicting tularemia with clinical, laboratory and demographical findings in the ED. *The American Journal of Emergency Medicine*, 34 (2), 218–221. https://doi.org/10.1016/j.ajem.2015.10.034

Ågren, A. & Lidberg, W. (2020). Markfuktighetsdata – Soil moisture data Digital, . https://www.skogsstyrelsen.se/sjalvservice/karttjanster/geodatatjanster/ftp/

Ågren, A.M., Larson, J., Paul, S.S., Laudon, H. & Lidberg, W. (2021). Use of multiple LIDAR-derived digital terrain indices and machine learning for high-resolution national-scale soil moisture mapping of the Swedish forest landscape. *Geoderma*, 404, 115280. https://doi.org/10.1016/j.geoderma.2021.115280

Appendix 1

List of equipment for field study and laboratory work.

Water collection

- GPS
- Camera
- Waders
- Protocol
- 2 watering cans
- A fine masked kitchen strainer
- 2 funnels, fitting the jugs
- Ultra-fine nylon mesh for straining
- 2 empty water drums à 20 l

Filtering

- Masterflex® E/S™ portable sampling drive, model no. 07571-00 07571-05, with recommended Masterflex® pump head installed
- Car battery with cables and adapter for EU-standard plug
- 56 pieces of ultra-filters, Asahi Kasei REXEED 25S
- 2 meters of Masterflex® L/S® 36 (9.5 mm) tube + 5 cm for splicing
- 30 cm of Masterflex® L/S® 24 (6.4 mm) tube
- 1 Masterflex® luer fitting that connects the L/S 24 tube with the ultra-filters
- A pressure gauge (0~2 bar) with adapter fitting 9.5 mm tube
- Pipe clips fitting 9.5 mm tube
- Stand and clamp for setting up the ultra-filters
- Screwdriver
- Paper and scissors for labeling samples
- Bucket
- 8 l plastic bags for filtered samples + small cable ties to seal bags
- Tarpaulin in case of rain

Decontamination and protection

- Tap water (2 l per sample)
- Distilled water (1 l per sample)
- HCl, 0.2 molar (~ 0.3 l per sample)
- Ethanol, surface disinfection 70 %
- Biohazard waste bags
- Disposable gloves and face masks
- Paper towels
- Hand disinfection

Eluent contains saline and two stock solutions:

Stock 1 (gives 30 ml solution, good for 60 samples)
3 ml tween (100%)
0.3 ml Silicon anti-foaming agent (100%)
26.7 ml distilled H_2O
Stock 2 (gives 30 ml solution, good for 60 samples)
3 g $NaPO_3$
30 ml distilled H_2O
Recipe for one sample:
0.5 l saline solution
0.5 ml stock 1
0.5 ml stock 2

Equipment

- Safety cabinet class II
- Centrifuge fit for falcon 50 ml tubes
- Masterflex® L/S® stationary drive with recommended Masterflex® pump head installed

Outside of safety cabinet:

- 56 x 0.5 l capped bottles for eluate (1 per sample).
- Cap fitting 0.5 l bottles with a centered hole measuring Ø 6,4 mm, and a smaller hole for air.
- 1 x 1 l bottle for eluent
- 168 x Falcon 50 ml tubes (3 per sample)
- 336 x Eppendorf tubes 2 ml (6 per sample)
- Masterflex® L/S® 24 (6.4 mm) tube: 1 x 100 cm, connected to ~7 cm of Masterflex® L/S® 36 (9.5 mm) tube + 1 x 40 cm, connected to a Masterflex® luer fitting (connecting the L/S® 24 tube with the ultra-filters) using x-small cable ties.
- 56 collected samples in ultra-filters (Asahi Kasei REXEED 25S)
- Filter pipette tips (1 ml)
- Pipette tips (1 ml)
- Paper towels
- Eluent

Inside of safety cabinet:

- Masterflex® L/S® stationary drive
- Falcon 50 ml tube containing 10-50 ml saline solution
- Stand and clamp for setting up the ultra-filters
- Eppendorf pipette, 100-1000 µl
- Tube rack for >10st falcon 50 ml tubes
- Ethanol 70% for disinfection
- Vortex

Appendix 2

GPS coordinates for sampling sites, given in SWEREF99/ RT90 2.5 gon V.

X-coordinate	Y-coordinate	Z (elevation)	Time	Sample ID	Water	depth	tularemia
1539395.32309827	6719751.50998489	109.462242	2021-08-03 08:38	1_1	Beaver system	biofilm	negative
1539395.32309828	6719751.50998490	109.462243	2021-08-04 08:38	1_2	Beaver system	surface	negative
1536726.52362822	6716142.56065081	99.134590	2021-08-03 15:13	1_3	Lake	biofilm	negative
1536726.52362823	6716142.56065082	99.134591	2021-08-04 15:13	1_4	Lake	surface	negative
1539650.44653441	6671469.7528254	55.653564	2021-08-04 09:44	2_1	Beaver system	biofilm	negative
1539650.44653442	6671469.7528255	55.653565	2021-08-05 09:44	2_2	Beaver system	surface	negative
1533623.8607711	6672619.70161293	98.523094	2021-08-04 14:35	2_3	Lake	biofilm	negative
1533623.8607712	6672619.70161294	98.523095	2021-08-05 14:35	2_4	Lake	surface	negative
1507800.32093853	6602647.88222761	20.676319	2021-08-05 09:16	3_1	Beaver system	biofilm	positive
1507800.32093854	6602647.88222762	20.676320	2021-08-06 09:16	3_2	Beaver system	surface	positive
1514265.11711249	6596016.26834629	27.083984	2021-08-05 13:44	3_3	Lake	biofilm	negative
1514265.11711250	6596016.26834630	27.083985	2021-08-06 13:44	3_4	Lake	surface	negative
1486205.05183475	6652299.49980284	205.657761	2021-08-06 08:50	4_1	Beaver system	biofilm	negative
1486205.05183476	6652299.49980285	205.657762	2021-08-07 08:50	4_2	Beaver system	surface	negative
1481118.37365342	6648014.4776108	181.107391	2021-08-06 14:56	4_3	Lake	biofilm	negative
1481118.37365343	6648014.4776109	181.107392	2021-08-07 14:56	4_4	Lake	surface	negative
1453260.1914426	6684132.94041062	173.851868	2021-08-08 07:46	5_1	Beaver system	biofilm	positive
1453260.1914427	6684132.94041063	173.851869	2021-08-09 07:46	5_2	Beaver system	surface	positive
1455143.1108954	6681755.69407611	173.484131	2021-08-08 12:02	5_3	Lake	biofilm	negative
1455143.1108955	6681755.69407612	173.484132	2021-08-09 12:02	5_4	Lake	surface	negative
1430395.43058386	6766245.82059898	37.392078	2021-08-09 10:17	6_1	Beaver system	biofilm	negative
1430395.43058387	6766245.82059899	37.392079	2021-08-10 10:17	6_2	Beaver system	surface	negative
1433658.52364013	6770645.1785546	135.454987	2021-08-09 14:58	6_3	Lake	biofilm	negative
1433658.52364014	6770645.1785547	135.454988	2021-08-10 14:58	6_4	Lake	surface	negative
1484695.912733	6828096.82641672	286.391327	2021-08-10 09:09	7_1	Beaver system	biofilm	negative
1484695.912734	6828096.82641673	286.391328	2021-08-11 09:09	7_2	Beaver system	surface	negative
1489005.91693917	6830546.99185607	345.805054	2021-08-10 12:27	7_3	Lake	biofilm	negative
1489005.91693918	6830546.99185608	345.805055	2021-08-11 12:27	7_4	Lake	surface	negative
1736441.80304852	7145316.09095572	193.853485	2021-08-12 10:41	8_1	Beaver system	biofilm	negative
1736441.80304853	7145316.09095573	193.853486	2021-08-13 10:41	8_2	Beaver system	surface	negative
1740708.18152899	7142103.98137148	100.287018	2021-08-12 14:03	8_3	Lake	biofilm	negative
1740708.18152900	7142103.98137149	100.287019	2021-08-13 14:03	8_4	Lake	surface	negative
1741588.43131673	7162215.96278286	103.312050	2021-08-13 09:58	9_1	Beaver system	biofilm	negative
1741588.43131674	7162215.96278287	103.312051	2021-08-14 09:58	9_2	Beaver system	surface	negative
1735449.72160292	7165049.19164825	84.044601	2021-08-13 13:08	9_3	Lake	biofilm	negative
1735449.72160293	7165049.19164826	84.044602	2021-08-14 13:08	9_4	Lake	surface	negative
1764066.79921667	7276004.72230707	74.861893	2021-08-14 10:37	10_1	Beaver system	biofilm	negative
1764066.79921668	7276004.72230708	74.861894	2021-08-15 10:37	10_2	Beaver system	surface	negative
1767757.23935419	7270229.19894789	-1.685436	2021-08-14 15:04	10_3	lake	biofilm	negative

1767757.23935420	7270229.19894790	-1.685437	2021-08-15 15:04	10_4	lake	surface	negative
1797679.66106392	7323640.04291345	16.630909	2021-08-16 07:33	11_1	Beaver system	biofilm	negative
1797679.66106393	7323640.04291346	16.630910	2021-08-17 07:33	11_2	Beaver system	surface	negative
1799640.36196429	7323847.5229558	1.335358	2021-08-16 12:11	11_3	Lake	biofilm	negative
1799640.36196430	7323847.5229559	1.335359	2021-08-17 12:11	11_4	Lake	surface	negative
1733943.23453403	7288050.80614383	181.221008	2021-08-17 08:34	12_1	Beaver system	biofilm	negative
1733943.23453404	7288050.80614384	181.221009	2021-08-18 08:34	12_2	Beaver system	surface	negative
1733037.11552892	7284865.53060103	223.086563	2021-08-17 11:38	12_3	Lake	biofilm	negative
1733037.11552893	7284865.53060104	223.086564	2021-08-18 11:38	12_4	Lake	surface	negative
1774653.29948149	7313792.31837491	22.232716	2021-08-18 09:31	13_1	Beaver system	biofilm	negative
1774653.29948150	7313792.31837492	22.232717	2021-08-19 09:31	13_2	Beaver system	surface	negative
1771123.58682413	7313681.80720095	-26.371046	2021-08-18 12:54	13_3	Lake	biofilm	negative
1771123.58682414	7313681.80720096	-26.371047	2021-08-19 12:54	13_4	Lake	surface	negative
1744032.99131578	7256483.49230637	469.677032	2021-08-19 09:16	14_1	Beaver system	biofilm	positive
1744032.99131579	7256483.49230638	469.677033	2021-08-20 09:16	14_2	Beaver system	surface	positive
1744710.99038849	7254729.42375003	207.063416	2021-08-19 12:19	14_3	Lake	biofilm	negative
1744710.99038850	7254729.42375004	207.063417	2021-08-20 12:19	14_4	Lake	surface	negative

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