**4 | DISCUSSION**

Our goal was to investigate the estimate of prevalence of the black spot disease across multiple spatial levels. This approach allowed us to skim over spatial patterns of infection, sampling methods bias and potential predictors of prevalence of infection in fish communities.

First, our results shows that prevalence of infection is spatially heterogenous in our system (Figure 3 and 4). Across the landscape, lakes present varying values of prevalence and estimates also vary within lakes. However, we did not find evidence of geographical patterns. A larger number of lakes sampled might allow to confirm whether infection is spatially random or whether patterns could not be detected because of our small sample size. Accordingly, none of the spatial attributes that we tested were good predictors of infection in the fish communities at small spatial scale (site-scale).

\*Examples de geographical patterns – number of lakes that were sampled

**[Comments on methods]**

Fish-host have been the focus of many field studies on aquatic parasites. However, sampling design and methods bias are not usually discussed although they can induce major bias in conclusions derived from wildlife samples. [eg.] Here, we investigated the relationship between the prevalence and the sampling effort across the landscape and, address the importance of the sampling method on prevalence estimate at lake and landscape-scale. Our data support method-induced bias in prevalence estimates at lake-scale and landscape-scale. At landscape-scale, prevalence estimates varied between 19.2% and 35.6 % (table Sx) depending on the method while we reported variations up to 46.7% between methods at lake-scale (table Sxy). Sampling wildlife is challenging, and all sampling method comes with their advantages and inconveniences. ……

Sampling effort must be sufficient to measure a representative biological metric in natural systems. Our results showed that low sampling effort lean toward overestimating the prevalence value at landscape-scale. [what is that?]. As shown in Figure 5, the minimum number of samples needed to adequately estimate landscape prevalence differed between methods. The only sampling method that reached a stable value was the observational snorkeling transects (around 10 samples) although the seine and methods combination showed only 1% of difference between simulated prevalence and real prevalence after 35 samples (table Sx). Indeed, this method allows observations on much more individuals than the seine and minnow traps explaining why it reaches stability faster. However, quality of observations underwater relies on, meteorological conditions, water transparency (i.e., color, turbidity) and expertise of the observers to identify fish species while moving. While fishing methods require a more extensive sampling, evaluation of infection is much more precise because the observer can take more time to look at fish individuals. Moreover, because fish must enter voluntarily in the trap, minnow traps are typically selective on bold and curious behavior. Even if seine nets are not directly behavior-selective devices, they come with habitat-selection restrictions like no large obstacles (e.g., branches, trunks, boulders) or shallow sites (sites must not be deeper than the height of the net). Thus, because species have different habitat preferences and that preferences can change in context of competition and/or predation, seine net might be indirectly selective on fish identity and behavior.

**[Comments on spatial patterns]**

\*Methods change lakes prevalence to. Sample diff. communities

\* Frequency distributionns

**[Comments on predictors]**

At the site-scale, we used GAMMs to inspect relationships between the prevalence estimate and environmental predictors (Figure 5). Results evidence some non-linear patterns supporting the use of flexible approach to model infection dynamics. We found that all physico-chemistry parameters (turbidity, temperature, dissolved oxygen, conductivity and pH) were good predictors of infection. Since, water physico-chemistry measurements do not require a lot of equipment and time compared to field assessment of fish infection, these results are promising for future monitoring and managing strategies of freshwater fish parasite infection. Consequently, we encourage researchers to investigate these relationships with other parasites and other locations/types of habitats.

Contrary to our initial believe, habitat structure does not seem to be an important factor driving prevalence of infection in fish communities from the littoral zone. However, we found a negative relationship between the macrophyte cover and the prevalence in our transect sites. The direction of this relationship is not what we had expected from previous studies where fishes from vegetated zones have been suggested to be more heavily infected (Hartmann & Nümann, 1977; Marcogliese et al., 2001; Ondrackova et al., 2004). On one hand, considering that cercariae have a short life-span (Combes et al., 1994; Pietrock & Marcogliese, 2003), encounter barriers might be structuring elements of infection dynamics in fish-host. As a results, dense macrophyte cover might prevent cercariae from encountering their fish-host. For instance, inert seaweed have been shown to obstruct transmission of trematodes cercariae to their mussel host (Prinz et al., 2009). Buss et al., (2022) also suggested that microplastic fibers might reduce success of infection by impeding cercariae to encounter its amphibian host. On the other hand, infected snails releasing cercariae in our lake system might not live or feed on macrophyte and thus, macrophyte cover would not be correlated to snail abundance and consequently parasite abundance. For instance, the mud amnicola *Amnicola limosus* is second intermediate host to at least one species of the black spot guild in our lake system (Levet, unpublished). This freshwater snail live partially in the sediment from the littoral zone (Pinel-Alloul & Magnin, 1973) and often graze on periphyton (Kesler, 1981) thus has no direct relationship with macrophyte cover.

Our results suggest that community structure is a driving factor of prevalence of infection by the black spot disease at small spatial scale. We found negative relationships for total fish abundance, non-host abundance and Simpson’s diversity index. Expectedly, total fish abundance and non-host abundance are positively correlated (Figure Sxx) indicating that the driving processes behind these relationships are probably the same. These results are expected by the dilution effect hypothesis. For trematode cercariae, a higher fish abundance means yes more encounter probability, but the proportion of parasites against the fish-host is diminished. As a result, prevalence would decrease with fish abundance if parasite abundance were not correlated to fish abundance. A higher abundance of non-host fish also means that success of infection by cercariae are lower, diminishing prevalence values. This has been observed both in the field and in experimental design [Refs]. Finally, diverse communities in our system tend to be less infected than communities with dominant species. In our lake system, *L. gibbosus* is a dominant species of fish communities but also the species with the highest prevalence of infection. Thus, presence of competitor species (e.g., *P. flavescens*) showing a lesser prevalence of infection might decrease the overall infection at the community level. Also, as previously discussed, presence of non-host species increases the diversity index, but might lower the prevalence in the community. In fact, non-host species can reduce infection success of cercariae by damaging or feeding on them(). Many animal species are known to partially feed on cercariae in aquatic systems such as zooplankton, clams, macroinvertebrates, cnidarian, and small fish (Kaplan et al., 2009; E. Mironova et al., 2019; K. Mironova et al., 2020; Schotthoefer et al., 2007; Vielma et al., 2019).