# Analysis of Environmental Factors that Correlate with the Presence of *Leptospiraceae* in a Native Hawaiian Mariculture System

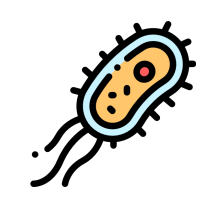Keanu Rochette-Yu Tsuen[1], Nalani Olguin[2], Hi'ilei Kawelo[4], Keli'i Kotubetey[4], Rosie Alegado[2,3]

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## Introduction

- Hawai'i has the **highest incidence of leptospirosis** in the United States, but cases of the disease are likely to **remain undiagnosed** or unreported.
- Leptospirosis is a **zoonosis caused by *Leptospira interrogans***, transmitted via environmental dissemination through the urine of mammal carriers (rats, feral swine).
- Gene *lipL32* is conservative across **pathogenic strains** of *Leptospira* and responsible for the pathogenicity of the bacteria.

## Hypothesis

We hypothesized that the **prevalence** of *Leptospiraceae* in the He'eia fishpond (HFP) positively **correlates** with significant **rainfall**, the **rainy season** of Hawai'i and **freshwater**.



Figure 1: In He'eia watershed, traditional agro-ecological sites, channelized streams (A) and established populations of feral swine may facilitate the transmission of the disease (B).

## Methods

- **Water sampling** every two weeks in 2014 - 2015 and every quarter in 2017 - 2019
- **DNA extraction** and sequencing of 16S rDNA gene. Clustering of **16S amplicon sequence variants (ASV)** to identify the spatial and temporal distribution of *Leptospiraceae*.
- **Salinity** data, measured using a YSI during water sampling; **precipitation** data, retrieved from the Hawai'i Climate Data Portal.
- **Distance** from sample sites **to river mouth** was obtained using the measuring tool in **Google Earth Pro.**
- Data analysis and visualization performed using **RStudio** and **QGIS**.

## Results : Precipitation

- *Leptospiraceae* presence is **significantly higher** in coastal waters during the **wet season** of Hawai'i.
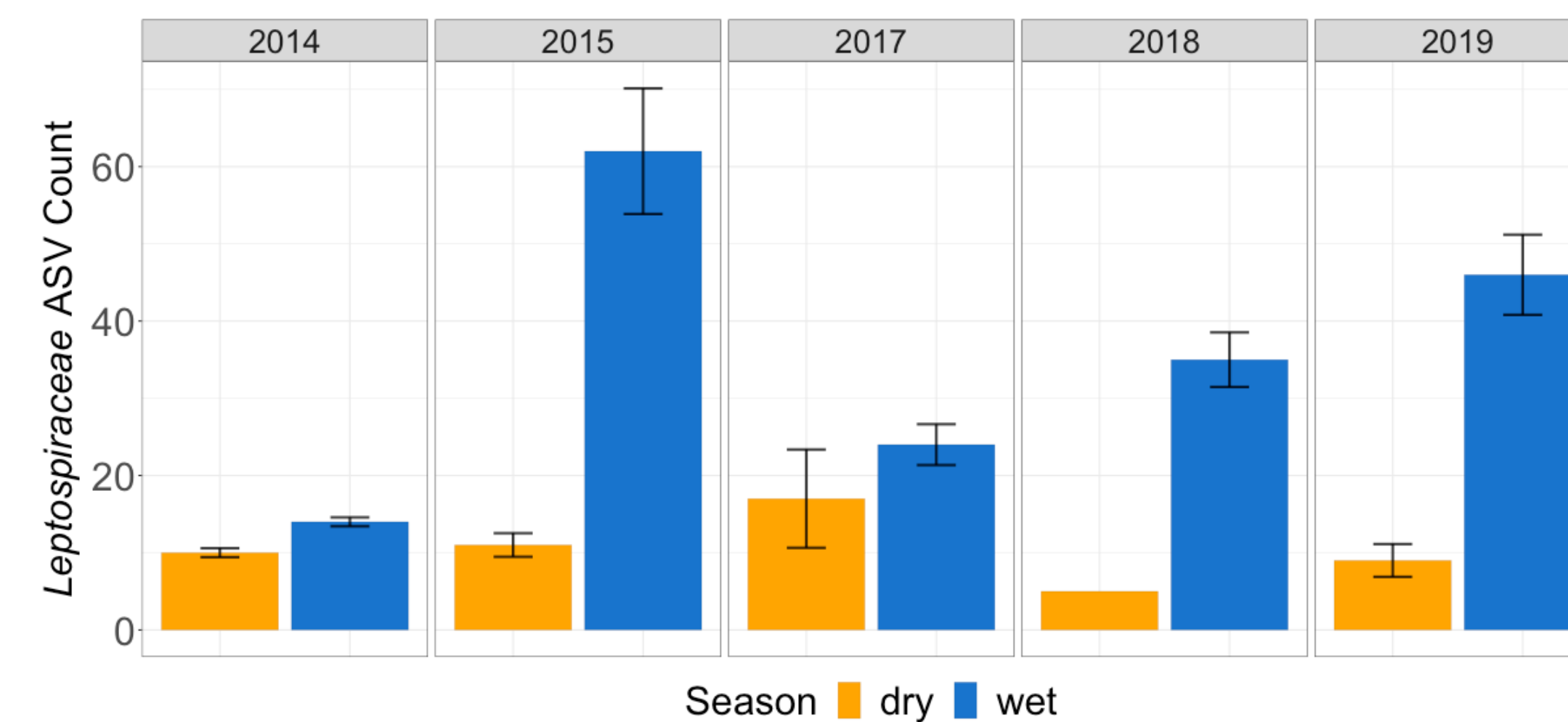- *Leptospiraceae* are **strongly correlated** with precipitation during **storm** conditions.



Figure 2: Comparison of the total number of *Leptospiraceae* ASV between the dry and wet seasons (2014-2015 and 2017-2019).



Figure 3: Variation of total ASV count between seasons (2014-2015 and 2017-2019).



**A** — 2014 - 2015 Timeseries
$y = 4.58 - 0.0317\,x$
$R^2 = 0.07, n = 12, P = 0.412$
Baseline

**B** — 2014 - 2015 Timeseries
$y = -0.243 + 0.0386\,x$
$R^2 = 0.64, n = 5, P = 0.102$
Storm conditions

Figure 4: Correlation between *Leptospiraceae* ASV and precipitation in non-storm (A) and storm (B) conditions.

## Results : Salinity

- Salinity varies and total *Leptospiraceae* count vary by season.
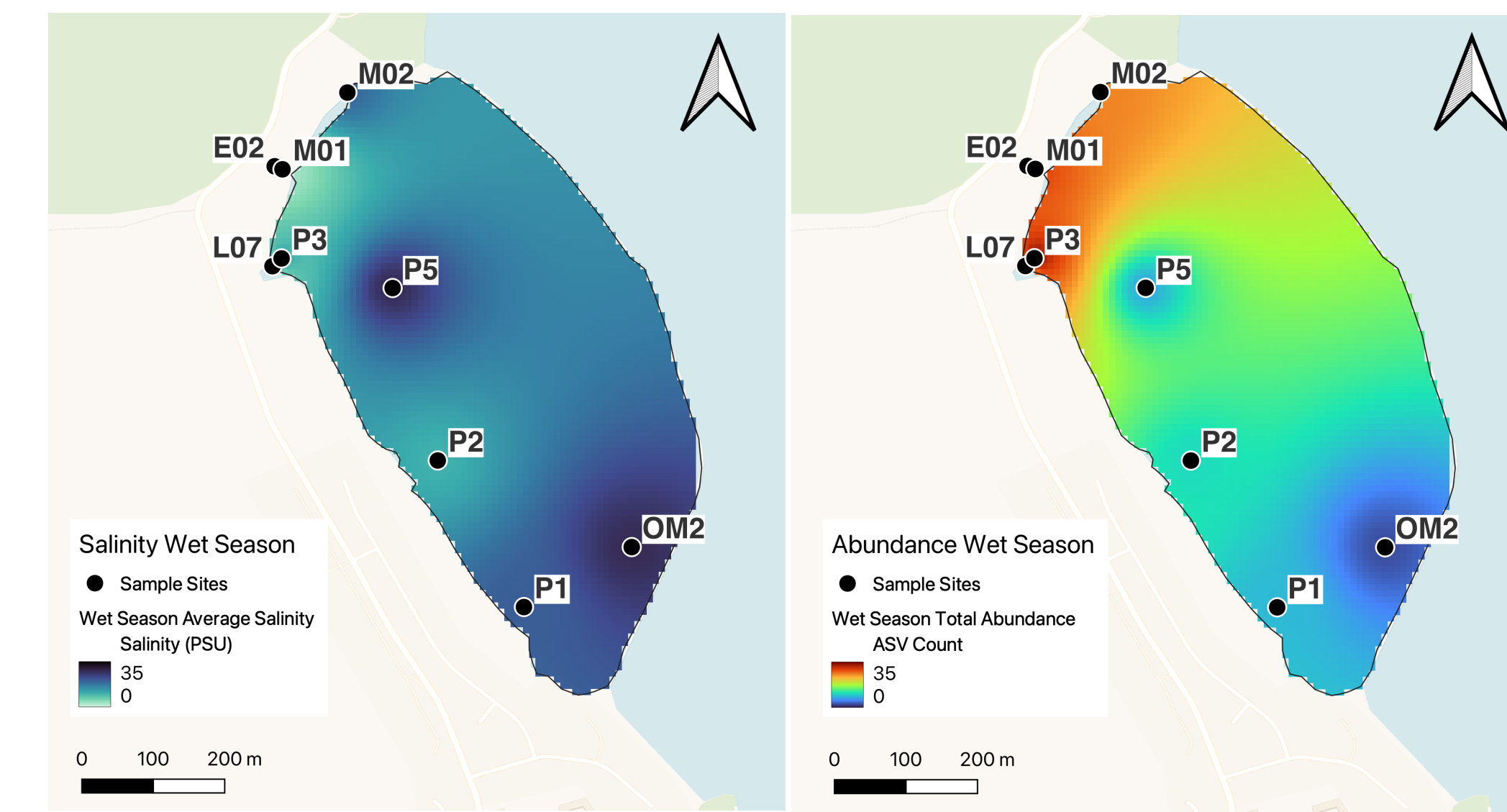- *Leptospiraceae* is **poorly correlated** to **salinity**, indicating a confounding variable.



Figure 5: Average salinity (left) and total abundance of *Leptospiraceae* (right) IDW map of He'eia fishpond during the wet season.



Figure 6: Average salinity (left) and total abundance of *Leptospiraceae* (right) IDW map of He'eia fishpond during the dry season.



**A** — 2014 - 2015 Timeseries
$y = 19.5 - 0.598\,x$
$R^2 = 0.64, n = 10, P = 0.006$

**B** — 2017 - 2019 Timeseries
$y = 7.39 + 0.0924\,x$
$R^2 = 0.02, n = 17, P = 0.561$

Figure 7: Correlation between salinity and the presence of *Leptospiraceae* in He'eia fishpond (2014-2015 : A; 2017-2019: B).

## Riverine Input of *Leptospiraceae*



$y = 28.6 - 0.0408\,x$
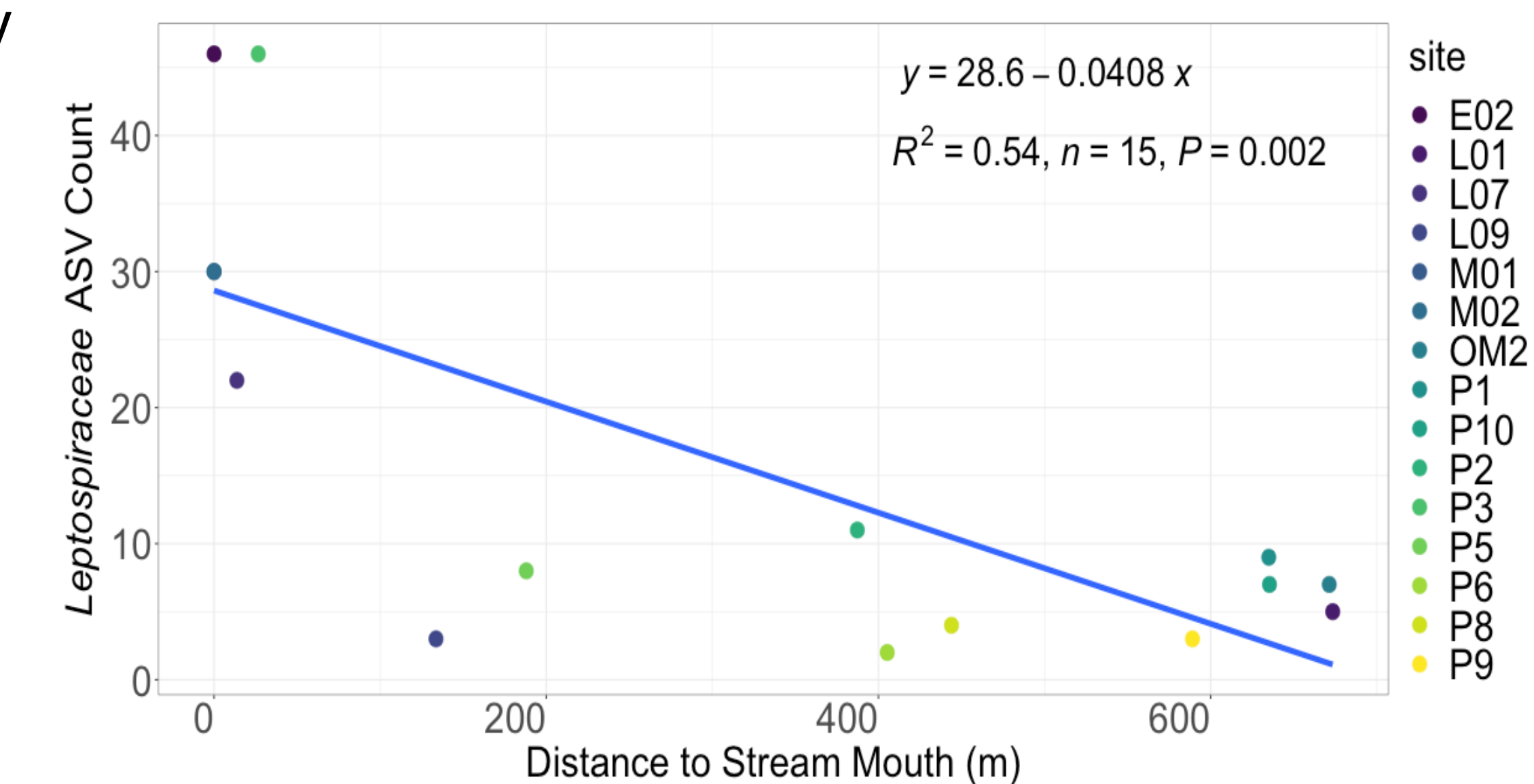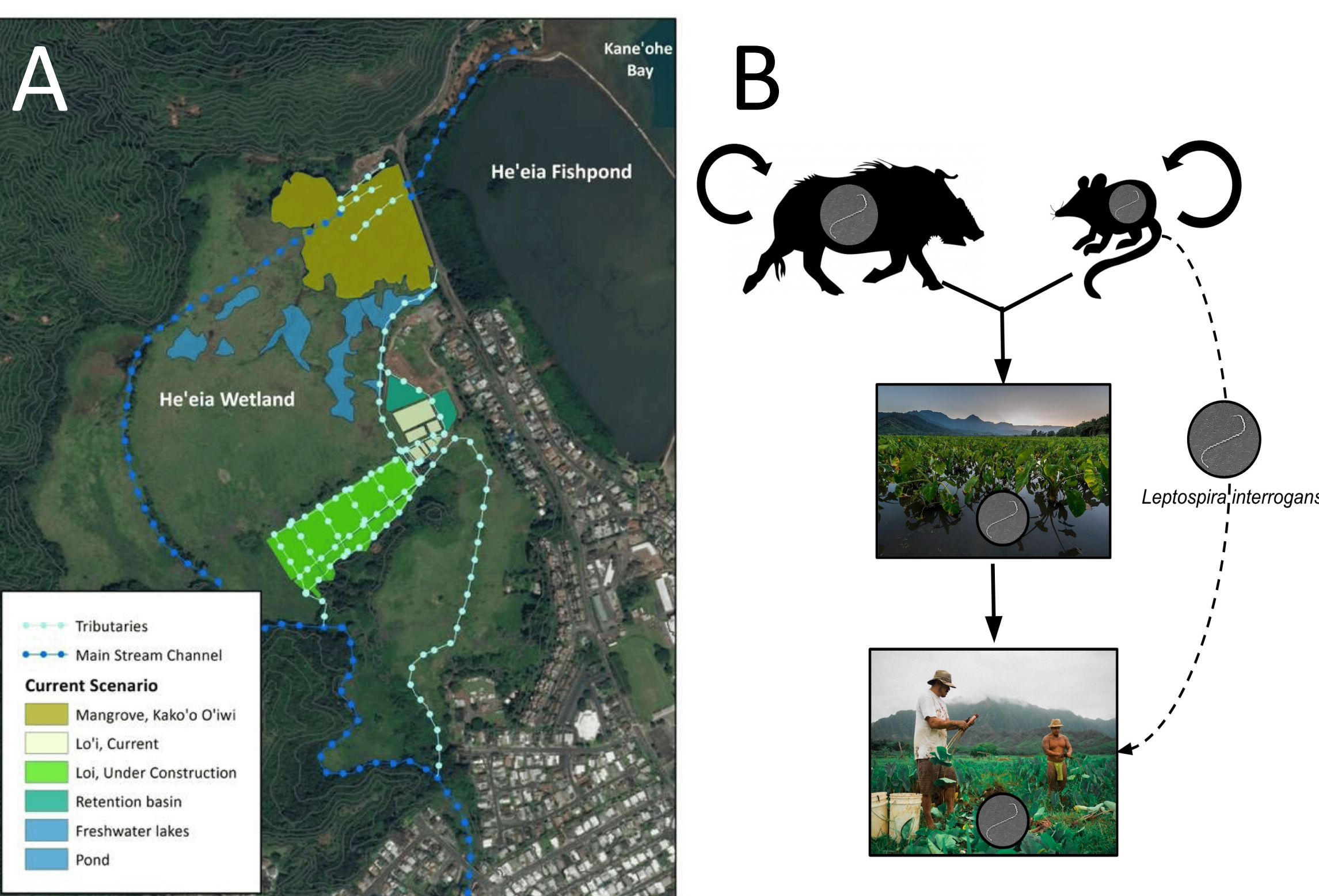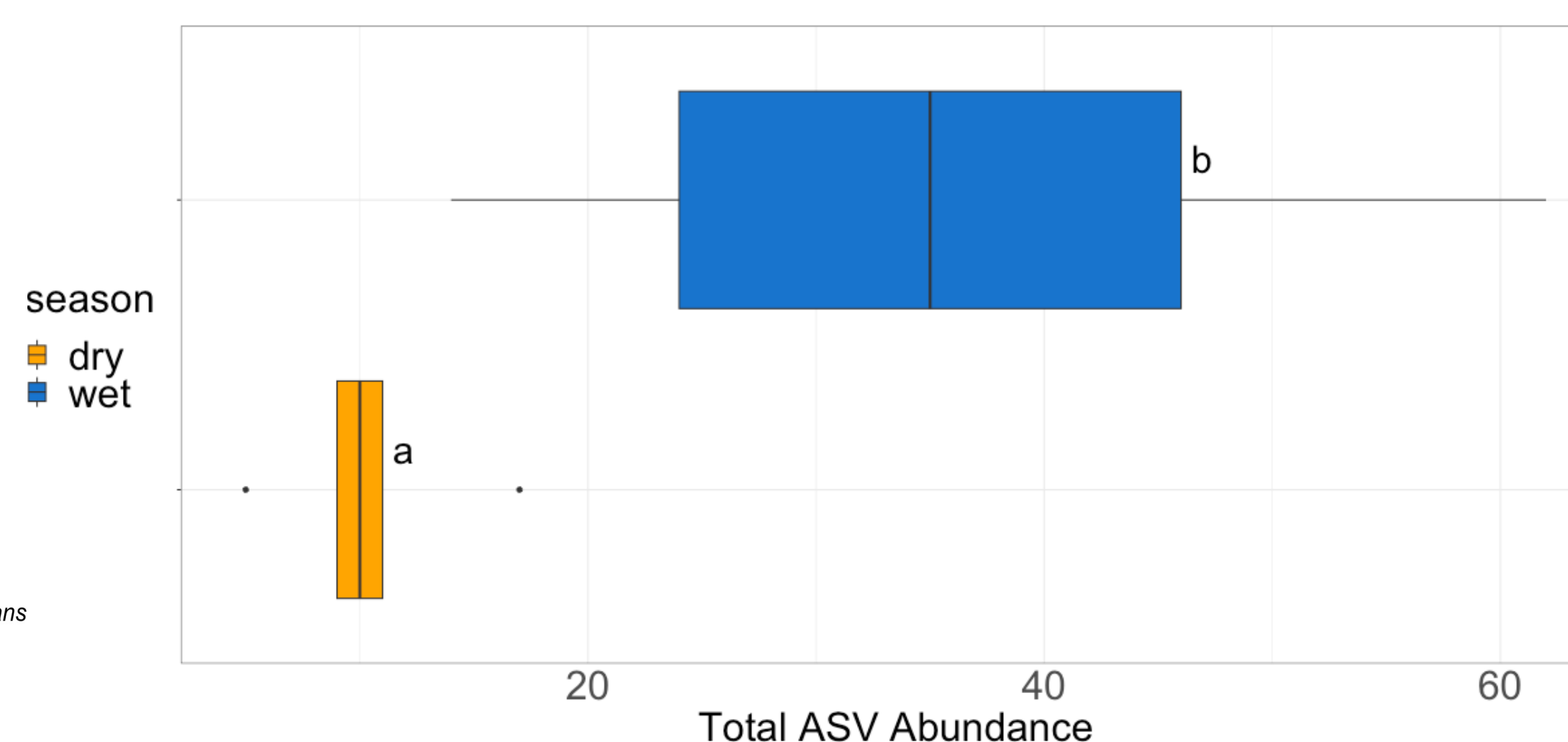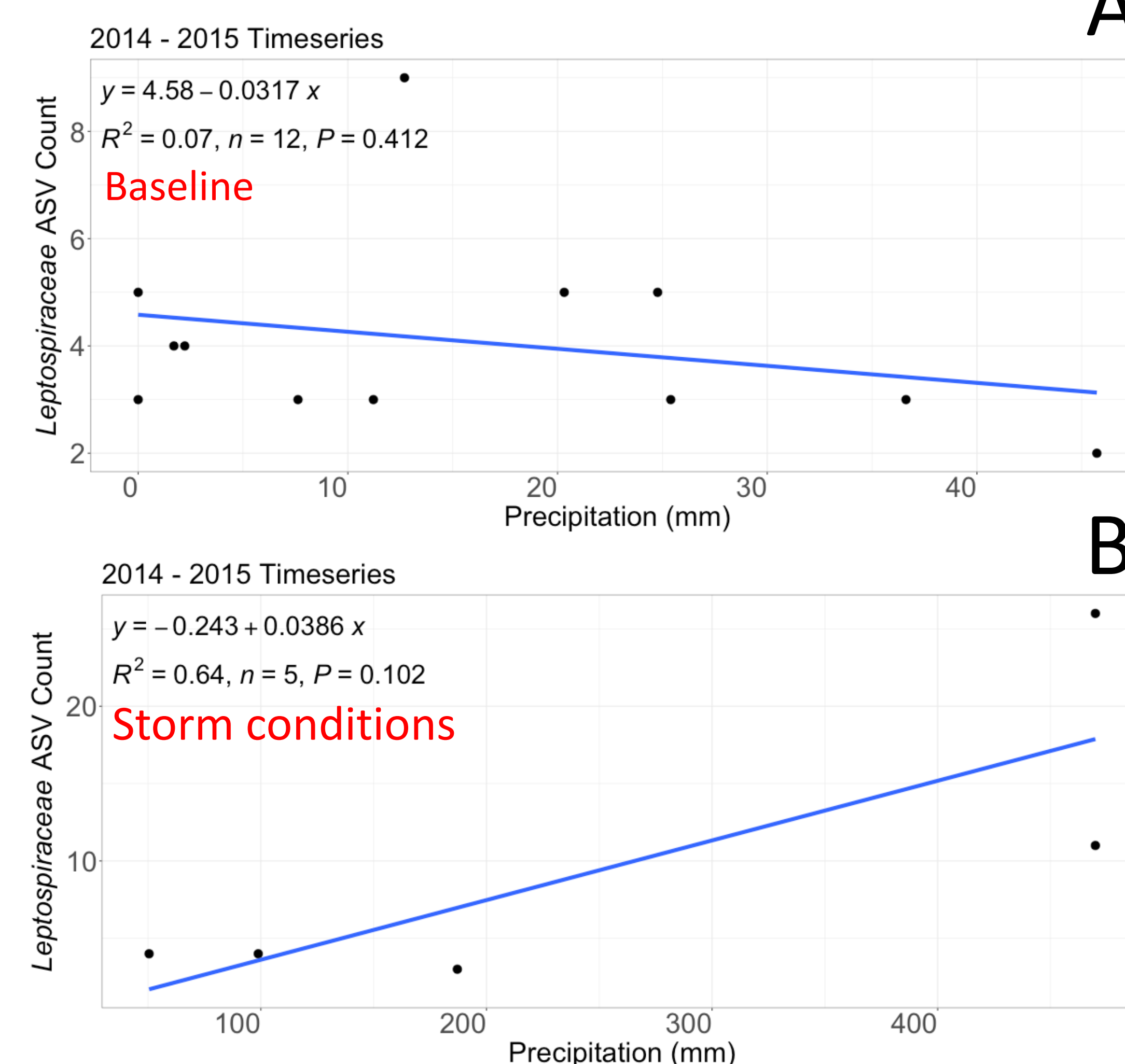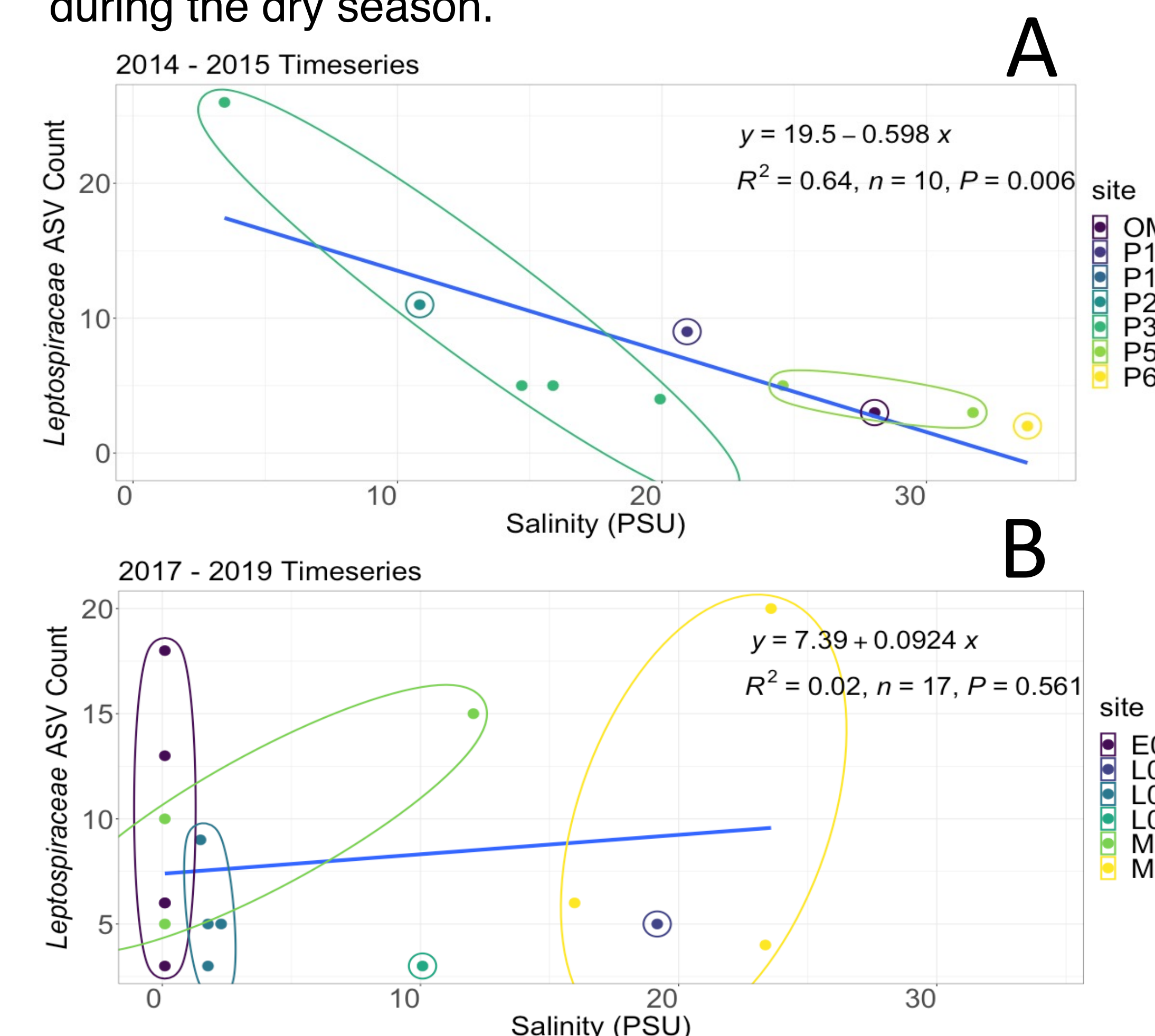$R^2 = 0.54, n = 15, P = 0.002$

Figure 8: Good correlation between distance to stream mouth (m) and total ASV indicate a riverine input of *Leptospiraceae*.

## Discussion

- *Leptospiraceae* were **significantly** more detected during the **wet season** and are **strongly associated** with **storm events.**
- Total *Leptospiraceae* ASV was **weakly correlated** with **salinity** in He'eia fishpond, but **strongly correlated** to the proximity with **He'eia stream**, suggesting a riverine input.
- This project helps to **develop methods** to understand the **circulation** of *Leptospiraceae* at the **watershed level** and inform local communities.
- **Additional data** showed the **absence of pathogenic *Leptospira*** in He'eia coastal water.

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