

Tick abundance Analysis

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Lets load our packages first

```
library(tidyverse)
library(tidyr)
library(ggplot2)
library(emmeans)
library(multcompView)
library(multcomp)
library(glmmTMB)
```

Introduction

Despite current efforts vector borne diseases like Lyme disease, transmitted in the western United States by the *Ixodes pacificus* tick, are emerging with greater frequency [1]. In humans, Lyme disease causes long term chronic arthritic, cardiac and dermatologic manifestations [2]. Assessing vector borne disease risk and understanding the web of transmission remains important for identifying stages in the transmission cycle where the vector and pathogen can be disrupted to reduce abundance in the environment. Disease risk is difficult to predict in the western United States, where *I. pacificus* feeds on a diverse set of vector meal hosts with contrasting effects on the disease risk [3]. *I. pacificus* has three host-seeking life stages where it can acquire *Borrelia burgdorferi* through horizontal transmission (i.e., from a reservoir host, not the parents) [4]. During its larval and nymphal stage, *I. pacificus* feeds primarily on small rodents and lizards, including the western fence lizard, dusky-footed woodrat, deer mouse, and western gray squirrel [5], [6]. Each species has different habitat preferences: *N. fuscipes* favor dense oak woodlands, while *P. maniculatus* occupy a broader range of habitats from woodlands to grasslands⁷. These two species are the primary reservoir hosts for *B. burgdorferi* through which *I. pacificus* can become infected. *S. occidentalis*, the preferred meal host of *I. pacificus*, is abundant in areas where there is sufficient basking habitat⁸. This species poses an interesting variable in the transmission cycle because it clears *I. pacificus* from the *B. burgdorferi* infection; however, it contributes to maintenance of high *I. pacificus* densities [6].

Ecotones are areas of ecological transition that experience rapid changes in abiotic conditions and community composition⁹. These unique conditions present in ecotones can influence the distribution of vector meals in the environment defining the epidemiological landscape [10]. However, little work has been done assessing disease risk along ecotonal communities in California. Therefore, for this study my aims will be to: 1) Understand differences in tick density between a oak woodland and annual grassland, habitat gradient. 2) Understand differences in infection prevalence between a oak woodland and annual grassland, habitat gradient. I expect to observe the greatest infection prevalence in oak woodlands, and the lowest in ecotone communities. Conversely, I expect tick densities to be greatest in ecotone communities and lower in oak woodland. Understanding how vector hosts and ticks behave on habitat edges is important in the face of human expansion and land use change [1]. Fragmentation of habitats can lead to greater human interaction between humans and wildlife through the displacement of species and the expansion of

the urban-wildlife interface¹¹. The study here will investigate how vectors and disease dynamics behave at habitat edges. However, it will also inform how species will respond to fragmentation, the creation of new habitat boundaries and edges between natural habitats and human settlements.

References

[1] Swei, A. et al. 2020. Vector Borne Zoonotic Dis. [3] Seltzer, E.G. et al. 2000. JAMA. [3] McDonald, A.J. et al. 2016. Ticks and Tick-borne Diseases. [4] Kwan, J.Y. et al. 2017. Mol Ecol. [5] Lane, R.S. et al. 2005. J Med Entomol. [6] Swei, A. et al. 2011. Proc Biol Sci. [7] Fellers, G. M. et al. 1994. Southwest Nat. [8] Swei, A. et al. 2011. Oecologia. [9] Marañón, T. et al. 1994. Madroño. [10] Despommier, D. et al. 2006. Ecohealth. [11] Lawrence, A. et al. 2018. Ecosphere.

Aim 1: Compare nymphal *I. pacificus* density among the forest edge, forest interior, and grasslands habitats.

Null Hypothesis: There are equal abundances of *Ixodes pacificus* in forest interiors, forest edge, and grasslands

Alternative Hypothesis: The abundances of *Ixodes pacificus* will be highest at the forest edge, intermediate at the forest interior and smallest in grasslands.

Dataset description:

This Data set contain plot level summaries of the ectoparasites found in each plot. Each row represents a plot that was surveyed. Each row contain the count of ticks of each species and each life stage that were found. As well as environmental characteristics including grass height, leaf litter density, and canopy cover, as well as the data in which samples were collected.

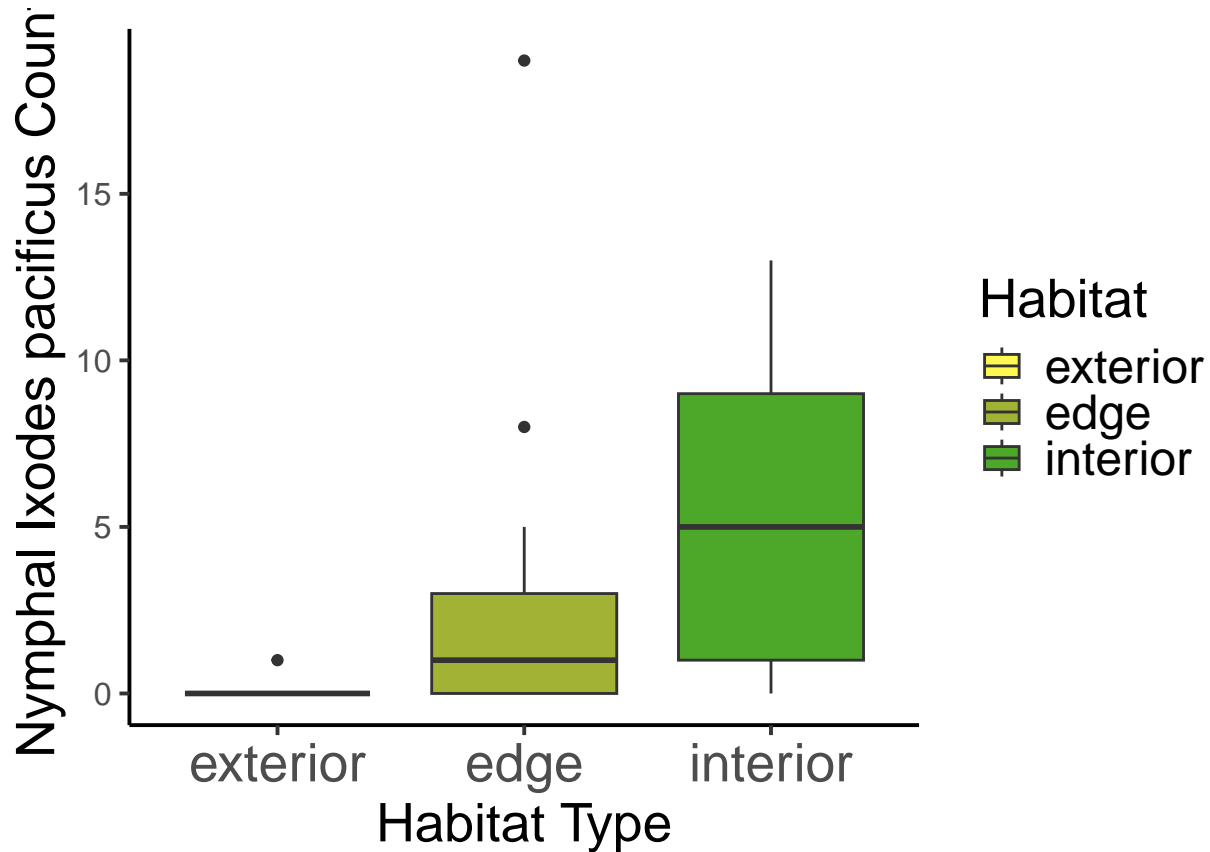
Lets read in our data

```
ticks<-read.csv("Dataset/Plot_Data copy.csv", header = TRUE, stringsAsFactors = TRUE)
```

Make a boxplot for IPACs per habitat

```
ticks$Habitat <- factor(ticks$Habitat, levels = c("exterior", "edge", "interior"))

p1 <- ggplot(ticks,aes(x=Habitat,y=IPAC_N, fill = Habitat)) +
  geom_boxplot() +
  theme_classic(15) +
  ylab("Nymphal Ixodes pacificus Count") +
  xlab("Habitat Type")+
  scale_fill_manual(values = c("exterior" = "#FEF851", "edge" = "#A1B234", "interior" = "#4DA829"))+
  theme(
    axis.title.x = element_text(size = 20),
    axis.title.y = element_text(size = 20),
    legend.title = element_text(size = 20), # Title of the legend
    axis.text.x = element_text(size = 20), # x-axis category labels
    legend.text = element_text(size = 18) # Labels inside the legend
  )
p1
```



```
p2 <- ggplot(ticks,aes(x=Habitat,y=IPAC_L, fill = Habitat)) +
  geom_boxplot() +
  theme_classic(15) +
  ylab("Larval Ixodes pacificus Count") +
  xlab("Habitat Type")+
  scale_fill_manual(values = c("exterior" = "#FEF851", "edge" = "#A1B234", "interior" = "#4DA829"))+
  theme(
    axis.title.x = element_text(size = 20),
    axis.title.y = element_text(size = 20),
    legend.title = element_text(size = 20), # Title of the legend
    axis.text.x = element_text(size = 20), # x-axis category labels
    legend.text = element_text(size = 18) # Labels inside the legend
  )
#p2

p3 <- ggplot(ticks,aes(x=Habitat,y=IPAC_A, fill = Habitat)) +
  geom_boxplot() +
  theme_classic(15) +
  ylab("Adult Ixodes pacificus Count") +
  xlab("Habitat Type")+
  scale_fill_manual(values = c("exterior" = "#FEF851", "edge" = "#A1B234", "interior" = "#4DA829"))+
  theme(
    axis.title.x = element_text(size = 20),
    axis.title.y = element_text(size = 20),
    legend.title = element_text(size = 20), # Title of the legend
    axis.text.x = element_text(size = 20), # x-axis category labels
  )
```

```

    legend.text = element_text(size = 18)      # Labels inside the legend
  )
#p3

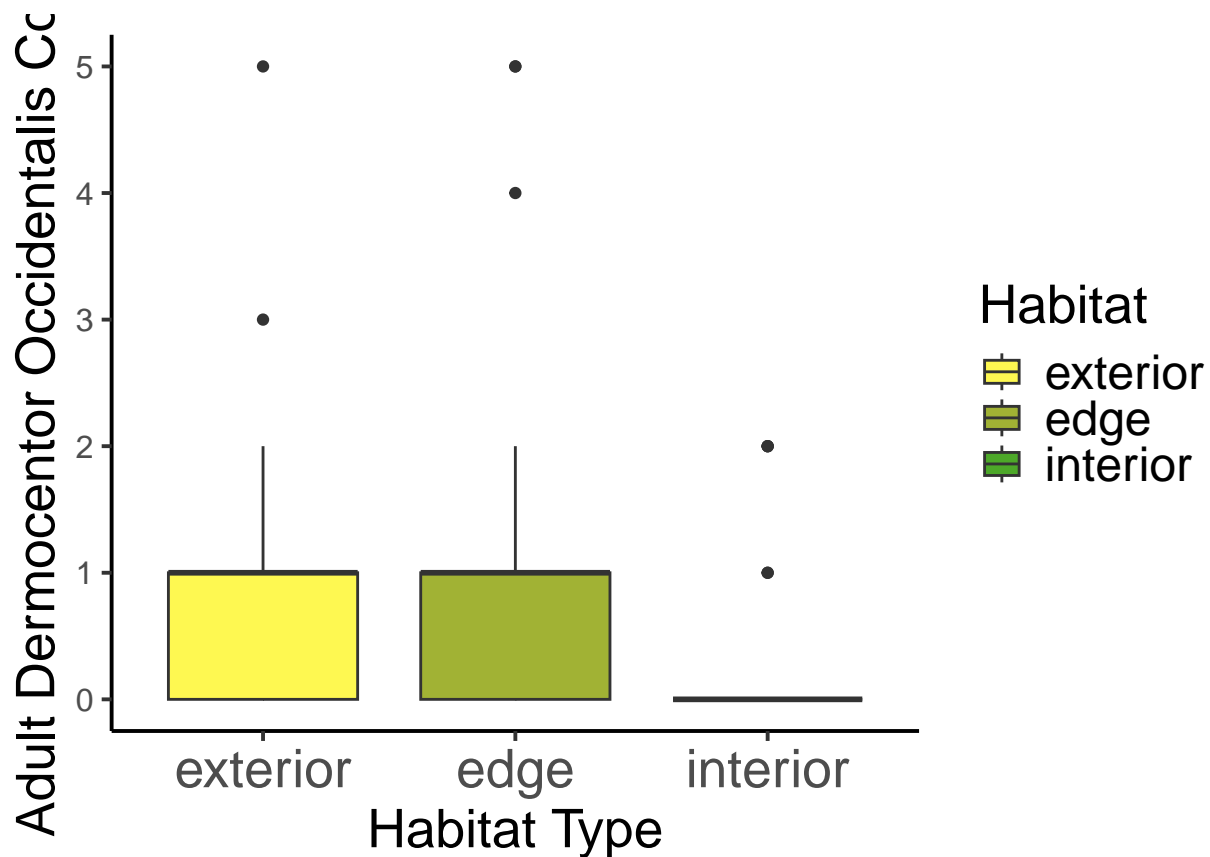
```

Make a boxplot & violin plot for DEOC Adults

```

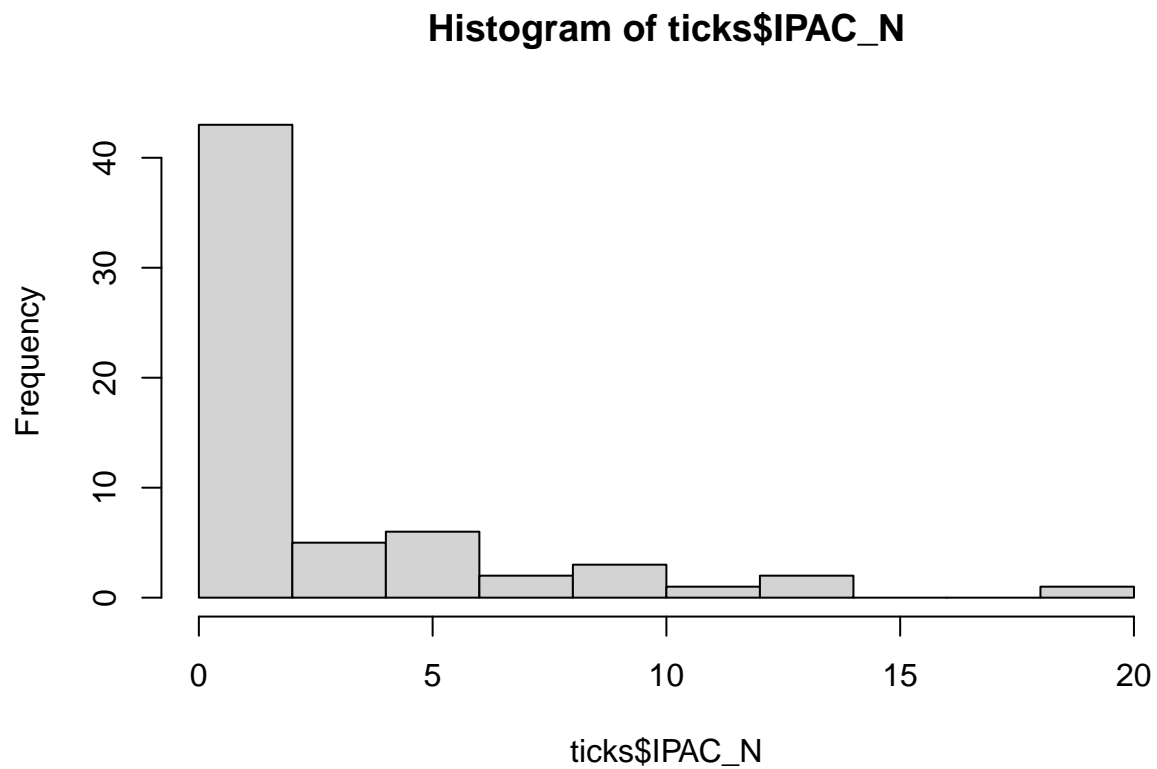
p4 <- ggplot(ticks,aes(x=Habitat,y=DEOC_A, fill = Habitat)) +
  geom_boxplot() +
  theme_classic(15) +
  ylab("Adult Dermocentor Occidentalis Count") +
  xlab("Habitat Type")+
  scale_fill_manual(values = c("exterior" = "#FEF851", "edge" = "#A1B234", "interior" = "#4DA829"))+
  theme(
    axis.title.x = element_text(size = 20),
    axis.title.y = element_text(size = 20),
    legend.title = element_text(size = 20), # Title of the legend
    axis.text.x = element_text(size = 20), # x-axis category labels
    legend.text = element_text(size = 18)  # Labels inside the legend
  )
p4

```



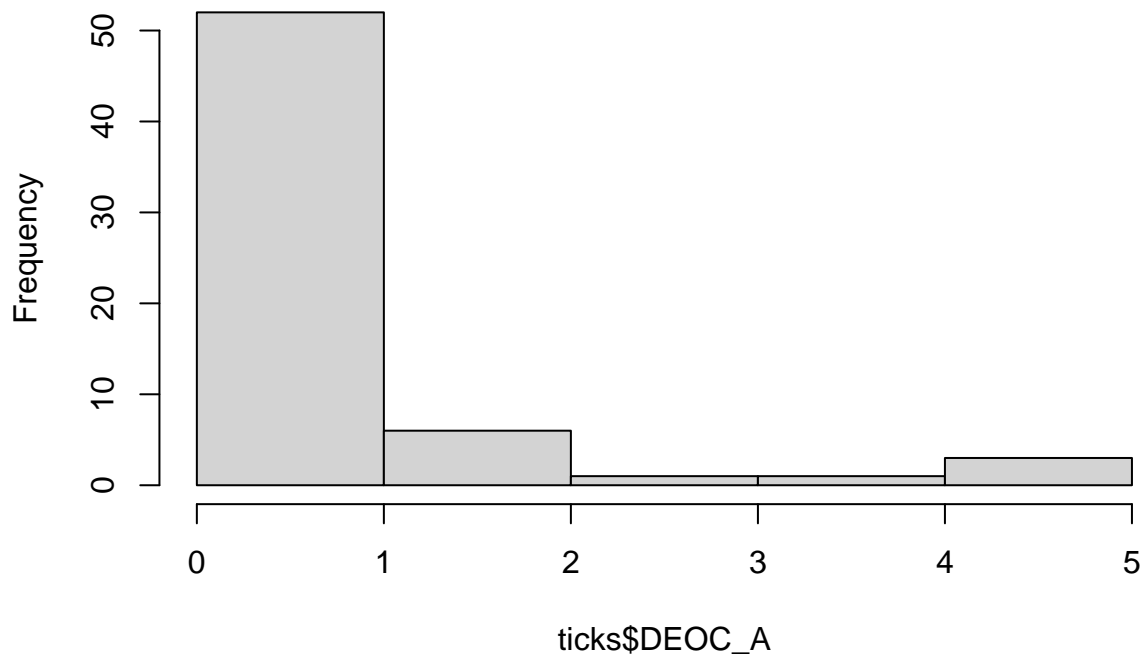
Lets look at the assumptions. First lets check the distribution using a histogram.

```
#hist(ticks$IPAC_L)  
hist(ticks$IPAC_N)
```



```
hist(ticks$DEOC_A)
```

Histogram of ticks\$DEOC_A



Statistical approach.

Both of these are skewed to the right and are count data so that means we must use a Generalized Linear Mixed Model (GLMM) and we can use a poisson family.

Lets start with IPAC Nymphs. We'll test diffent models with habitat as our fixed effect, and test site and date or a combination of both as our random effects

```
m4<- glmmTMB(IPAC_N ~ Habitat +  
              (1 | Site),  
              family = poisson(link = "log"),  
              data = ticks)  
m5<- glmmTMB(IPAC_N ~ Habitat +  
              (1 | Site) + (1 | Date),  
              family = poisson(link = "log"),  
              data = ticks)  
m6<- glmmTMB(IPAC_N ~ Habitat,  
              family = poisson(link = "log"),  
              data = ticks)
```

```
AIC(m4, m5, m6)
```

```
##      df      AIC  
## m4   4 301.7075
```

```
## m5 5 227.4870
## m6 3 299.9437
```

```
# We picked model five which has the best AIC Score
summary(m5)
```

```
## Family: poisson ( log )
## Formula:          IPAC_N ~ Habitat + (1 | Site) + (1 | Date)
## Data: ticks
##
##      AIC      BIC    logLik -2*log(L)  df.resid
##    227.5    238.2   -108.7    217.5      58
##
## Random effects:
##
## Conditional model:
## Groups Name      Variance Std.Dev.
## Site  (Intercept) 1.502e-09 3.875e-05
## Date  (Intercept) 1.135e+00 1.066e+00
## Number of obs: 63, groups: Site, 3; Date, 24
##
## Conditional model:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)    -3.639     1.033  -3.521  0.00043 ***
## Habitatedge      3.970     1.009   3.933  8.38e-05 ***
## Habitatinterior  5.088     1.013   5.025  5.04e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Lets repeat the same process but instead for *Dermocentor occidentalis*

```
m7<- glmmTMB(DEOC_A ~ Habitat +
              (1 | Site),
              family = poisson(link = "log"),
              data = ticks)
m8<- glmmTMB(DEOC_A ~ Habitat +
              (1 | Site) + (1 | Date),
              family = poisson(link = "log"),
              data = ticks)
m9<- glmmTMB(DEOC_A ~ Habitat,
              family = poisson(link = "log"),
              data = ticks)
```

```
AIC(m7, m8, m9)
```

```
##      df      AIC
## m7  4 163.8057
## m8  5 163.7918
## m9  3 167.5636
```

```
# We picked model 8 which has the best AIC Score
summary(m8)
```

```
## Family: poisson ( log )
## Formula:      DEOC_A ~ Habitat + (1 | Site) + (1 | Date)
## Data: ticks
##
##           AIC          BIC      logLik -2*log(L)  df.resid
##        163.8        174.5       -76.9     153.8       58
##
## Random effects:
##
## Conditional model:
## Groups Name      Variance Std.Dev.
## Site (Intercept) 0.2259   0.4753
## Date (Intercept) 0.2192   0.4681
## Number of obs: 63, groups: Site, 3; Date, 24
##
## Conditional model:
##           Estimate Std. Error z value Pr(>|z|)
## (Intercept)   -0.4008    0.3998  -1.002   0.3161
## Habitatedge     0.2877    0.3118   0.923   0.3562
## Habitatinterior -0.8094    0.4334  -1.868   0.0618 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Now lets run the emmeans for the models that we picked.

Lets start with IPAC Nymphs

```
library(emmeans)
em5 <- emmeans(m5, ~ Habitat)

# Pairwise comparisons between habitat types
pairs(em5, adjust = "tukey")
```

```
## contrast          estimate      SE df z.ratio p.value
## exterior - edge      -3.97 1.010 Inf  -3.933  0.0002
## exterior - interior  -5.09 1.010 Inf  -5.025  <.0001
## edge - interior     -1.12 0.211 Inf  -5.309  <.0001
##
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 3 estimates
```

Lets move on to DEOC Adults

```
library(emmeans)
em8 <- emmeans(m8, ~ Habitat)
```

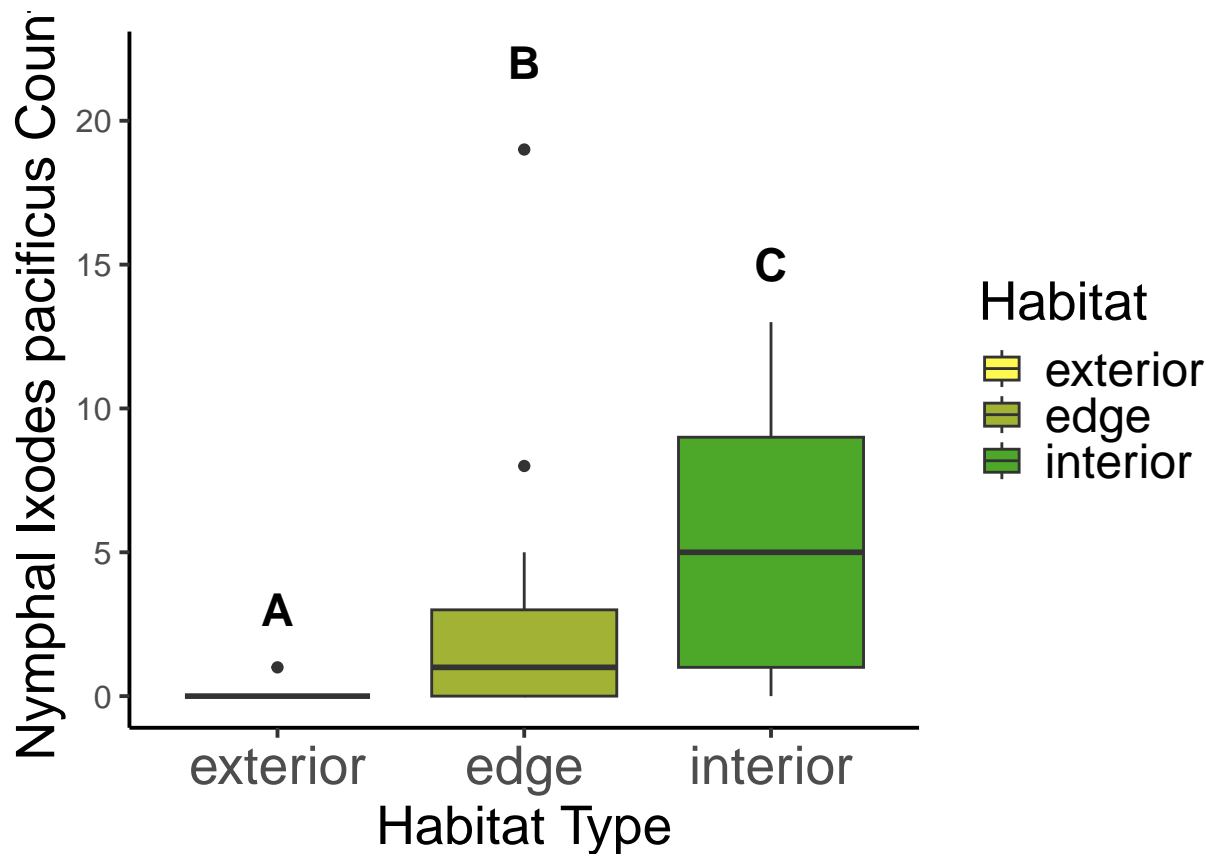


```
# Pairwise comparisons between habitat types
pairs(em8, adjust = "tukey")
```

```
## contrast      estimate      SE df z.ratio p.value
## exterior - edge    -0.288 0.312 Inf  -0.923  0.6259
## exterior - interior  0.809 0.433 Inf   1.868  0.1481
## edge - interior     1.097 0.417 Inf   2.631  0.0232
##
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 3 estimates
```

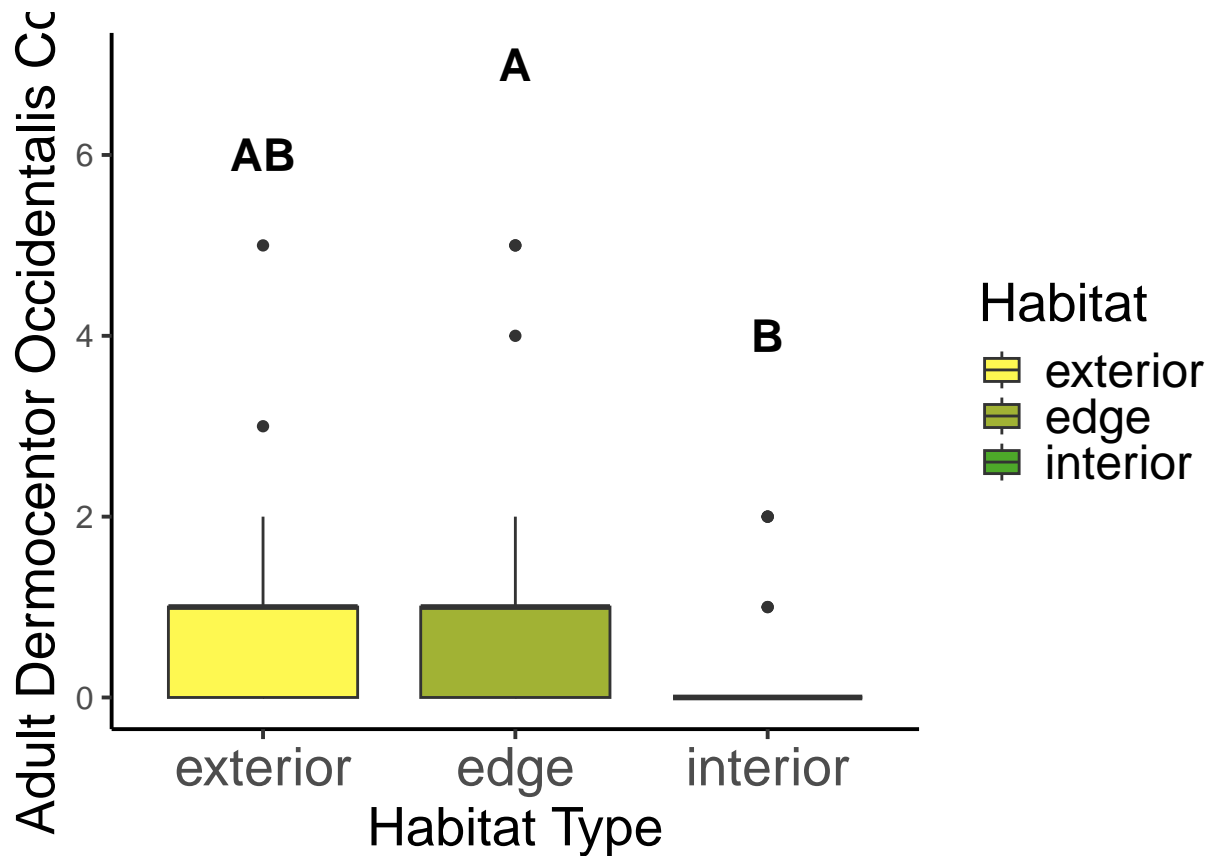
Finalize Figure for IPAC N

```
p1+ annotate("text", x = 1, y = 3, label = "A", size = 6, fontface = "bold") + annotate("text", x = 2, y = 20, label = "B", size = 6, fontface = "bold") + annotate("text", x = 3, y = 15, label = "C", size = 6, fontface = "bold")
```



Finalize Figure for DEOC adults

```
p4+ annotate("text", x = 1, y = 6, label = "AB", size = 6, fontface = "bold") + annotate("text", x = 2, y = 6, label = "AC", size = 6, fontface = "bold") + annotate("text", x = 3, y = 6, label = "BC", size = 6, fontface = "bold")
```



Results.

For nymphal *Ixodes pacificus* we observed that their populations increase as you move along a gradient from grassland to oakwoodlands. Each habitat surveyed had a significantly different abundance of ticks. For our *Dermocentor occidentalis* adults we observed the most abundances in grasslands (exterior) forest edge, while interior experienced the lowest abundances. This was in contradiction to our alternative hypothesis. This is a possible indication that *Ixodes pacificus* populations are more strongly influenced by environmental conditions compared to host availability. However, we still observed a significant amount of both *Dermocentor occidentalis* adults and *Ixodes pacificus* nymphs at the forest edge indicating that human development built adjacent to forests will be a potential point of connection between these vectors and human communities in the future. Both of these species are of epidemiological importance because they can transmit diseases like Lyme Disease and Pacific Coast fever.