08\_Class\_Activity

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# In class activity 8: Study Design and Power Analysis

## Introduction

This document demonstrates key concepts in experimental design using ecological examples, focusing on:

1. **Formulating research questions**
2. **Understanding different study designs**
3. **Recognizing proper replication vs. pseudoreplication**
4. **Designing appropriate controls**
5. **Conducting power analysis** (a priori and post hoc)
6. **Planning sampling strategies**

We’ll work with simulated pine needle data to practice these concepts.

Let’s start by exploring these concepts with hands-on examples!

# **Part 1:** Load Required Packages

# Load required packages
library(tidyverse) # For data manipulation and visualization

── Attaching core tidyverse packages ──────────────────────── tidyverse 2.0.0 ──
✔ dplyr 1.1.4 ✔ readr 2.1.5
✔ forcats 1.0.0 ✔ stringr 1.5.1
✔ ggplot2 3.5.2 ✔ tibble 3.3.0
✔ lubridate 1.9.4 ✔ tidyr 1.3.1
✔ purrr 1.1.0
── Conflicts ────────────────────────────────────────── tidyverse\_conflicts() ──
✖ dplyr::filter() masks stats::filter()
✖ dplyr::lag() masks stats::lag()
ℹ Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become errors

library(patchwork) # For combining plots
library(pwr) # For power analysis

# Set seed for reproducible results
set.seed(42)

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|  Package Overview |
| * **tidyverse**: Collection of packages for data science (includes ggplot2, dplyr, etc.)
* **patchwork**: Easily combine multiple ggplot2 plots
* **pwr**: Functions for power analysis and sample size calculations
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# **Part 2:** Formulating Research Questions

Before we design any study, we need clear research questions. Let’s practice with pine needle ecology.

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|  Activity 1: Research Question Practice |
| Think about pine trees on campus. Write down 2-3 specific research questions about:* - Pine needle characteristics (length, density, color)
* - Environmental factors (wind, sunlight, soil)
* - Tree health or growth

**Example questions:*** - Does wind exposure affect pine needle length?
* - Do pine needles on south-facing branches differ from north-facing branches?
* - Does tree size influence needle density?

**Your questions:**1. 1. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. 2. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
3. 3. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
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# **Part 3:** Understanding Study Design Types

Let’s simulate data for different types of studies to understand their strengths and limitations.

## Natural Experiment: Wind Exposure Study

# Simulate pine needle data from naturally exposed and sheltered locations
# This represents a "natural experiment" - we didn't manipulate wind exposure

# Create data for exposed locations (shorter needles due to wind stress)
exposed\_data <- data.frame(
 location = rep(paste0("Exposed\_", 1:5), each = 8),
 wind\_exposure = "exposed",
 needle\_length\_mm = rnorm(40, mean = 75, sd = 10),
 tree\_id = rep(1:5, each = 8)
)

# Create data for sheltered locations (longer needles, less wind stress)
sheltered\_data <- data.frame(
 location = rep(paste0("Sheltered\_", 1:5), each = 8),
 wind\_exposure = "sheltered",
 needle\_length\_mm = rnorm(40, mean = 90, sd = 12),
 tree\_id = rep(6:10, each = 8)
)

# Combine the datasets
natural\_exp\_data <- rbind(exposed\_data, sheltered\_data)

# Look at the first few rows
head(natural\_exp\_data)

 location wind\_exposure needle\_length\_mm tree\_id
1 Exposed\_1 exposed 88.70958 1
2 Exposed\_1 exposed 69.35302 1
3 Exposed\_1 exposed 78.63128 1
4 Exposed\_1 exposed 81.32863 1
5 Exposed\_1 exposed 79.04268 1
6 Exposed\_1 exposed 73.93875 1

# Visualize the natural experiment data
natural\_plot <- natural\_exp\_data %>%
 ggplot(aes(x = wind\_exposure, y = needle\_length\_mm, fill = wind\_exposure)) +
 geom\_boxplot(alpha = 0.7) +
 geom\_jitter(width = 0.2, alpha = 0.5) +
 labs(
 x = "Wind Exposure",
 y = "Needle Length (mm)")
natural\_plot



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|  Natural Experiments: Pros and Cons |
| * **Advantages:** - Realistic conditions - Large scale possible - Cost-effective
* **Disadvantages:** - Cannot control confounding variables - Cannot determine causation direction - Many unmeasured factors might influence results

**Question:** What other factors besides wind might differ between “exposed” and “sheltered” locations? |

## Manipulative Experiment: Controlled Wind Study

# Simulate a controlled experiment where we manipulate wind exposure
# All trees start similar, then we apply treatments

# Create data for control group (normal conditions)
control\_data <- data.frame(
 treatment = "control",
 needle\_length\_mm = rnorm(25, mean = 85, sd = 8),
 tree\_id = 1:25
)

# Create data for wind treatment (artificial wind exposure)
wind\_treatment\_data <- data.frame(
 treatment = "wind\_treatment",
 needle\_length\_mm = rnorm(25, mean = 78, sd = 8),
 tree\_id = 26:50
)

# Combine the datasets
manipulative\_data <- rbind(control\_data, wind\_treatment\_data)

# Visualize the manipulative experiment
manipulative\_plot <- manipulative\_data %>%
 ggplot(aes(x = treatment, y = needle\_length\_mm, fill = treatment)) +
 geom\_boxplot(alpha = 0.7) +
 geom\_jitter(width = 0.2, alpha = 0.5) +
 labs(
 x = "Treatment",
 y = "Needle Length (mm)") +
 theme\_minimal() +
 theme(legend.position = "none")

manipulative\_plot



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|  Manipulative Experiments: Key Features |
| **Advantages:** - Can establish causation - Control confounding variables - Random assignment eliminates bias**Disadvantages:** - Often smaller scale - May not reflect natural conditions - Can be expensive and logistically challenging**Key Question:** Which experiment gives stronger evidence for causation? |

# **Part 4:** Identifying Proper Replication

One of the most common mistakes in ecological studies is pseudoreplication. Let’s practice identifying true replication vs. pseudoreplication.

# Example 1: Pseudoreplication - multiple measurements from same trees
pseudo\_data <- data.frame(
 treatment = rep(c("fertilized", "control"), each = 20),
 tree\_id = rep(c("Tree\_A", "Tree\_B"), each = 20), # Only 2 trees total!
 needle\_length\_mm = c(
 rnorm(20, mean = 95, sd = 5), # Tree A (fertilized)
 rnorm(20, mean = 80, sd = 5) # Tree B (control)
 ),
 measurement = rep(1:20, times = 2)
)

# Example 2: True replication - multiple trees per treatment
true\_rep\_data <- data.frame(
 treatment = rep(c("fertilized", "control"), each = 20),
 tree\_id = paste0("Tree\_", 1:40), # 40 different trees
 needle\_length\_mm = c(
 rnorm(20, mean = 95, sd = 8), # 20 fertilized trees
 rnorm(20, mean = 80, sd = 8) # 20 control trees
 )
)

# Create comparison plots
pseudo\_plot <- pseudo\_data %>%
 ggplot(aes(x = treatment, y = needle\_length\_mm, fill = treatment)) +
 geom\_boxplot() +
 labs(title = "Pseudoreplication",
 subtitle = "Multiple needles from only 2 trees",
 x = "Treatment", y = "Needle Length (mm)") +
 theme\_minimal() +
 theme(legend.position = "none")

true\_plot <- true\_rep\_data %>%
 ggplot(aes(x = treatment, y = needle\_length\_mm, fill = treatment)) +
 geom\_boxplot() +
 labs(title = "True Replication",
 subtitle = "Multiple trees per treatment",
 x = "Treatment", y = "Needle Length (mm)") +
 theme\_minimal() +
 theme(legend.position = "none")

# Combine plots
pseudo\_plot + true\_plot



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|  Pseudoreplication Alert! |
| **Pseudoreplication occurs when:*** - You treat subsamples as independent when they’re not
* - Multiple measurements from the same experimental unit
* - Replication at wrong scale for your hypothesis

**Common examples:*** - Multiple leaves from one plant
* - Multiple samples from one lake or from one fish
* - Multiple plots within one treatment area

**Why it’s bad:*** - Underestimates variability
* - Inflates sample size artificially
* - Increases Type I error (false positives)
 |

## Activity: Identify Replication Issues

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|  Activity 2: Replication Practice |
| For each scenario, identify if there’s proper replication or pseudoreplication:**Scenario A:** Testing fertilizer effects by using 1 large pot with fertilizer containing 10 pine seedlings, and 1 control pot with 10 seedlings.- **Your answer:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_- **Fix:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**Scenario B:** Testing altitude effects by measuring needle length on 5 trees at 1000m elevation and 5 trees at 2000m elevation.- **Your answer:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_- **Fix:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**Scenario C:** Testing soil pH by measuring 20 needles each from 10 trees in acidic soil and 10 trees in basic soil.- **Your answer:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_- **Fix:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |

# **Part 5:** Power Analysis - Planning Your Study

Power analysis helps us determine how many samples we need to detect an effect if it really exists.

## A Priori Power Analysis (Before Data Collection)

# Scenario: We want to detect a difference in needle length between
# fertilized and control trees

# Based on pilot data, we expect:
control\_mean <- 80 # mm
fertilized\_mean <- 90 # mm
pooled\_sd <- 12 # mm

# Calculate effect size (Cohen's d)
effect\_size <- abs(fertilized\_mean - control\_mean) / pooled\_sd
cat("Effect size (Cohen's d):", round(effect\_size, 2), "\n")

Effect size (Cohen's d): 0.83

# Interpret effect size
if(effect\_size < 0.2) {
 interpretation <- "small"
} else if(effect\_size < 0.5) {
 interpretation <- "small-medium"
} else if(effect\_size < 0.8) {
 interpretation <- "medium-large"
} else {
 interpretation <- "large"
}
cat("This is a", interpretation, "effect size\n")

This is a large effect size

# Calculate required sample size for 80% power
power\_result <- pwr.t.test(
 d = effect\_size, # Effect size
 sig.level = 0.05, # Alpha level (significance)
 power = 0.8, # Desired power (80%)
 type = "two.sample" # Two-sample t-test
)

print(power\_result)

 Two-sample t test power calculation

 n = 23.60467
 d = 0.8333333
 sig.level = 0.05
 power = 0.8
 alternative = two.sided

NOTE: n is number in \*each\* group

cat("\nWe need", ceiling(power\_result$n), "trees per group for 80% power\n")

We need 24 trees per group for 80% power

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|  Understanding Effect Size (Cohen’s d) |
| * **d = 0.2**: Small effect (subtle difference)
* **d = 0.5**: Medium effect (moderate difference)
* **d = 0.8**: Large effect (substantial difference)

**Cohen’s d formula:** d = (Mean₁ - Mean₂) / Pooled Standard Deviation |

## Visualizing Power Curves

# Create a power curve showing relationship between sample size and power
sample\_sizes <- seq(5, 50, by = 2)

# Calculate power for each sample size
power\_values <- sapply(sample\_sizes, function(n) {
 power\_test <- pwr.t.test(n = n, d = effect\_size, sig.level = 0.05, type = "two.sample")
 return(power\_test$power)
})

# Create data frame for plotting
power\_df <- data.frame(
 sample\_size = sample\_sizes,
 power = power\_values
)

# Create power curve plot
power\_curve\_plot <- ggplot(power\_df, aes(x = sample\_size, y = power)) +
 geom\_line(color = "blue", size = 1.2) +
 geom\_hline(yintercept = 0.8, linetype = "dashed", color = "red", size = 1) +
 geom\_vline(xintercept = ceiling(power\_result$n), linetype = "dashed", color = "red", size = 1) +
 annotate("text", x = ceiling(power\_result$n) + 5, y = 0.5,
 label = paste("n =", ceiling(power\_result$n)), color = "red") +
 annotate("text", x = 35, y = 0.85, label = "80% Power", color = "red") +
 ylim(0, 1) +
 labs(title = "Power Analysis: Sample Size vs. Statistical Power",
 subtitle = paste("Effect size (d) =", round(effect\_size, 2)),
 x = "Sample Size (per group)",
 y = "Statistical Power") +
 theme\_minimal()

Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
ℹ Please use `linewidth` instead.

power\_curve\_plot



## Post Hoc Power Analysis (After Data Collection)

# Imagine we collected data with n = 15 per group but found no significant difference
# Was our study adequately powered?

observed\_n <- 15

# Calculate the power we actually had
actual\_power <- pwr.t.test(
 n = observed\_n,
 d = effect\_size,
 sig.level = 0.05,
 type = "two.sample"
)

print(actual\_power)

 Two-sample t test power calculation

 n = 15
 d = 0.8333333
 sig.level = 0.05
 power = 0.5962064
 alternative = two.sided

NOTE: n is number in \*each\* group

cat("\nWith n =", observed\_n, "per group, we only had",
 round(actual\_power$power \* 100, 1), "% power\n")

With n = 15 per group, we only had 59.6 % power

if(actual\_power$power < 0.8) {
 cat("This study was underpowered! A non-significant result might be due to insufficient sample size.\n")
} else {
 cat("This study had adequate power. A non-significant result likely reflects no true effect.\n")
}

This study was underpowered! A non-significant result might be due to insufficient sample size.

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|  Activity 3: Power Analysis Practice |
| **Scenario:** You want to study the effect of drought stress on pine needle length. Based on literature, you expect:* - Control trees: mean = 85mm, SD = 10mm
* - Drought-stressed trees: mean = 75mm, SD = 10mm

**Calculate the following:**# Your turn! Fill in the values and run the code# Step 1: Calculate effect sizecontrol\_mean <- 9drought\_mean <- 99 pooled\_sd <- 999effect\_size <- abs(control\_mean - drought\_mean) / pooled\_sdprint(paste("Effect size:", round(effect\_size, 2)))# Step 2: Calculate required sample size for 80% powerpower\_result <- pwr.t.test( d = effect\_size, sig.level = 0.05, power = 0.8, type = "two.sample")print(power\_result)print(paste("Required sample size:", ceiling(power\_result$n), "trees per group"))# Step 3: What if you can only collect 12 trees per group?limited\_power <- pwr.t.test( n = 12, d = effect\_size,  sig.level = 0.05, type = "two.sample")print(paste("Power with n=12:", round(limited\_power$power \* 100, 1), "%"))**Questions:** 1. What is the effect size for this drought study? 2. How many trees do you need per group for 80% power? 3. If you can only sample 12 trees per group, what power will you have? |

# **Part 6:** Sampling Design Strategies

Different research questions require different sampling approaches. Let’s explore the main types.

## Simple Random Sampling

# Simulate a campus with pine trees scattered randomly
set.seed(123)
campus\_trees <- data.frame(
 tree\_id = 1:100,
 x\_coordinate = runif(100, 0, 100), # Random x positions
 y\_coordinate = runif(100, 0, 100), # Random y positions
 needle\_length = rnorm(100, mean = 80, sd = 12)
)

# Simple random sampling: select 20 trees randomly
random\_sample\_ids <- sample(1:100, size = 20, replace = FALSE)
random\_sample <- campus\_trees[campus\_trees$tree\_id %in% random\_sample\_ids, ]

# Visualize sampling design
campus\_plot <- ggplot(campus\_trees, aes(x = x\_coordinate, y = y\_coordinate)) +
 geom\_point(color = "lightgreen", size = 2, alpha = 0.6) +
 geom\_point(data = random\_sample, color = "red", size = 3) +
 labs(title = "Simple Random Sampling",
 subtitle = "Red points = selected trees",
 x = "X Coordinate", y = "Y Coordinate") +
 theme\_minimal()

campus\_plot



## Stratified Sampling

# Simulate campus with different zones (north vs south)
set.seed(124)
stratified\_trees <- data.frame(
 tree\_id = 1:100,
 x\_coordinate = runif(100, 0, 100),
 y\_coordinate = runif(100, 0, 100),
 zone = ifelse(runif(100) > 0.5, "North", "South"),
 needle\_length = rnorm(100, mean = 80, sd = 12)
)

# Add zone effect to needle length
stratified\_trees$needle\_length[stratified\_trees$zone == "South"] <-
 stratified\_trees$needle\_length[stratified\_trees$zone == "South"] + 8

# Stratified sampling: sample equally from each zone
north\_trees <- stratified\_trees[stratified\_trees$zone == "North", ]
south\_trees <- stratified\_trees[stratified\_trees$zone == "South", ]

# Sample 10 from each zone
north\_sample <- north\_trees[sample(nrow(north\_trees), 10), ]
south\_sample <- south\_trees[sample(nrow(south\_trees), 10), ]
stratified\_sample <- rbind(north\_sample, south\_sample)

# Visualize stratified sampling
stratified\_plot <- ggplot(stratified\_trees, aes(x = x\_coordinate, y = y\_coordinate, color = zone)) +
 geom\_point(size = 2, alpha = 0.6) +
 geom\_point(data = stratified\_sample, size = 4, shape = 21, fill = "yellow", stroke = 2) +
 labs(title = "Stratified Sampling",
 subtitle = "Yellow outline = selected trees, equal sampling from each zone",
 x = "X Coordinate", y = "Y Coordinate", color = "Zone") +
 theme\_minimal()

stratified\_plot



## Systematic Sampling

# Systematic sampling along a transect
set.seed(125)
transect\_trees <- data.frame(
 tree\_id = 1:50,
 distance\_m = seq(0, 490, by = 10), # Trees every 10m along transect
 needle\_length = rnorm(50, mean = 80, sd = 10)
)

# Add distance effect (trees farther from road have longer needles)
transect\_trees$needle\_length <- transect\_trees$needle\_length +
 (transect\_trees$distance\_m \* 0.02)

# Systematic sampling: every 5th tree
systematic\_sample <- transect\_trees[seq(1, 50, by = 5), ]

# Visualize systematic sampling
systematic\_plot <- ggplot(transect\_trees, aes(x = distance\_m, y = 1)) +
 geom\_point(size = 3, alpha = 0.6, color = "lightblue") +
 geom\_point(data = systematic\_sample, size = 4, color = "red") +
 labs(title = "Systematic Sampling Along Transect",
 subtitle = "Red points = selected trees (every 5th tree)",
 x = "Distance from Road (m)", y = "") +
 theme\_minimal() +
 theme(axis.text.y = element\_blank(), axis.ticks.y = element\_blank()) +
 ylim(0.5, 1.5)

systematic\_plot



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|  Sampling Strategy Comparison |
| **Simple Random Sampling:*** - Best for: General population estimates
* - Pros: Unbiased, simple analysis
* - Cons: May miss important subgroups

**Stratified Sampling:*** - Best for: When you know there are distinct subgroups
* - Pros: Ensures representation of all strata
* - Cons: Requires prior knowledge of strata

**Systematic Sampling:*** - Best for: Studying gradients or patterns
* - Pros: Good spatial coverage, easy to implement
* - Cons: Risk of bias if there’s hidden periodicity
 |

# **Part 7:** Putting It All Together - Design Your Own Study

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|  Activity 4: Complete Study Design |
| **Research Question:** Does fertilizer application affect pine needle length?**Design your study by answering these questions:**1. **Study Type:** Will this be a natural experiment or manipulative experiment? Why?
	* Your answer: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. **Sample Size:** Using the following parameters, calculate required sample size:
	* Expected control mean: 80mm
	* Expected fertilized mean: 88mm
	* Expected SD for both groups: 10mm
	* Desired power: 80%

# Calculate effect size and sample size neededcontrol\_mean <- 80fertilized\_mean <- 88pooled\_sd <- 10effect\_size <- abs(fertilized\_mean - control\_mean) / pooled\_sdpower\_result <- pwr.t.test( d = effect\_size, sig.level = 0.05, power = 0.8, type = "two.sample")print(power\_result)1. **Controls:** What controls will you include? Consider both positive and negative controls.
	* Your answer: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. **Randomization:** How will you randomize tree assignment to treatments?
	* Your answer: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
3. **Replication:** How will you ensure proper replication? What would be pseudoreplication?
	* Proper replication: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
	* Pseudoreplication to avoid: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
4. **Independence:** What factors might violate independence? How will you address them?
	* Your answer: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
5. **Potential Confounds:** What other variables might affect needle length that you need to control for?
	* Your answer: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
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# **Part 8:** Analyzing Your Designed Study

Let’s simulate data from the study you designed and analyze it:

# Simulate data based on your study design
set.seed(200)

# Use the sample size you calculated (or use 20 if you didn't calculate)
n\_per\_group <- 20 # Replace with your calculated sample size

# Create the experimental data
study\_data <- data.frame(
 tree\_id = 1:(2 \* n\_per\_group),
 treatment = rep(c("control", "fertilized"), each = n\_per\_group),
 needle\_length\_mm = c(
 rnorm(n\_per\_group, mean = 80, sd = 10), # Control group
 rnorm(n\_per\_group, mean = 88, sd = 10) # Fertilized group
 )
)

# Calculate summary statistics
summary\_stats <- study\_data %>%
 group\_by(treatment) %>%
 summarise(
 n = n(),
 mean\_length = mean(needle\_length\_mm),
 sd\_length = sd(needle\_length\_mm),
 se\_length = sd\_length / sqrt(n)
 )

print(summary\_stats)

# A tibble: 2 × 5
 treatment n mean\_length sd\_length se\_length
 <chr> <int> <dbl> <dbl> <dbl>
1 control 20 79.3 7.85 1.76
2 fertilized 20 87.4 8.57 1.92

# Create visualization
study\_plot <- study\_data %>%
 ggplot(aes(x = treatment, y = needle\_length\_mm, fill = treatment)) +
 geom\_boxplot(alpha = 0.7) +
 geom\_jitter(width = 0.2, alpha = 0.6) +
 stat\_summary(fun = mean, geom = "point", shape = 23, size = 3, fill = "white") +
 labs(title = "Fertilizer Effect on Pine Needle Length",
 subtitle = "White diamonds show group means",
 x = "Treatment",
 y = "Needle Length (mm)") +
 theme\_minimal() +
 theme(legend.position = "none")

study\_plot



# Conduct statistical test
t\_test\_result <- t.test(needle\_length\_mm ~ treatment, data = study\_data)
print(t\_test\_result)

 Welch Two Sample t-test

data: needle\_length\_mm by treatment
t = -3.1164, df = 37.715, p-value = 0.003493
alternative hypothesis: true difference in means between group control and group fertilized is not equal to 0
95 percent confidence interval:
 -13.364541 -2.837295
sample estimates:
 mean in group control mean in group fertilized
 79.33243 87.43335

# Interpret results
if(t\_test\_result$p.value < 0.05) {
 cat("\nResult: Significant difference found!\n")
 cat("Fertilizer significantly affects needle length (p =",
 round(t\_test\_result$p.value, 4), ")\n")
} else {
 cat("\nResult: No significant difference found.\n")
 cat("No evidence that fertilizer affects needle length (p =",
 round(t\_test\_result$p.value, 4), ")\n")
}

Result: Significant difference found!
Fertilizer significantly affects needle length (p = 0.0035 )

# Calculate actual effect size observed
observed\_effect\_size <- abs(diff(t\_test\_result$estimate)) /
 sqrt(((n\_per\_group-1) \* var(study\_data$needle\_length\_mm[study\_data$treatment == "control"]) +
 (n\_per\_group-1) \* var(study\_data$needle\_length\_mm[study\_data$treatment == "fertilized"])) /
 (2\*n\_per\_group - 2))

cat("Observed effect size (Cohen's d):", round(observed\_effect\_size, 2), "\n")

Observed effect size (Cohen's d): 0.99

# **Summary and Key Takeaways**

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|  What We Learned Today |
| 1. **Study Design Matters:** Statistics cannot fix a poorly designed study
2. **Replication:** Must be at the appropriate scale for your research question
3. **Controls:** Essential for ruling out alternative explanations
4. **Power Analysis:** Plan your sample size before collecting data
5. **Sampling Strategy:** Choose the approach that best fits your research question
6. **Integration:** Good analysis flows naturally from good design

**Remember:*** - Design before you collect data
* - Consider practical and logistical constraints
* - Be transparent about limitations
* - Correlation ≠ causation (especially in natural experiments)
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|  Common Pitfalls to Avoid |
| 1. **Pseudoreplication:** Taking multiple measurements from the same experimental unit
2. **Inadequate Power:** Collecting too few samples to detect meaningful effects
3. **Poor Controls:** Not controlling for important confounding variables
4. **Non-random Sampling:** Introducing bias through convenience sampling
5. **HARKing:** Hypothesizing After Results are Known

**The Golden Rule:** Plan your analysis when you plan your experiment! |