RESEARCH ARTICLE

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A Statistical Comparison of Box Quadrat, Open Quadrat and Transect Sampling Techniques for Accurate Diversity and Abundance Assessment of Oedipodinae Grasshopper Community

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ABSTRACT

Accurate monitoring of grasshopper populations is vital for understanding ecosystem health and for the formulation of conservation strategies. This research compares the efficacy of three sampling techniques such as Box Quadrat, Open Quadrat, and Transect for assessing grasshopper diversity and abundance in the grasslands of Sindh Agriculture University, Tando Jam, Pakistan, over a period from May to September 2023. A total area of 40m² was surveyed using each method to evaluate their effectiveness in capturing species richness and population density. These results reveal that the method is superior in terms of capturing species diversity, identifying 25% more species compared to the Box Quadrat and 15% more than the Open Quadrat. The Transect method also demonstrated the highest statistical significance in estimating population density, with a 20% lower coefficient of variation compared to the other methods. In contrast, the Box Quadrat method showing good precision in density estimates, identified four species overall, indicating a trade-off between precision and species diversity. The Open Quadrat method, with its moderate variability, provided intermediate results but was less effective in consistently capturing rare species. Findings highlight the Transect method's effectiveness in providing a comprehensive view of grasshopper populations and their spatial distribution. The Box Quadrat method's precision and the Open Quadrat method's flexibility offer valuable alternatives depending on specific research goals and habitat characteristics. This study contributes to refining grasshopper monitoring techniques and offers practical recommendations for enhancing ecological surveys and conservation planning.

Keywords: Grasshopper Monitoring, Sampling Techniques, Species Diversity, Population Density, Ecological Surveys, Conservation Strategies

INTRODUCTION

Research on grasshopper populations across different habitats is vital for ecological assessments and conservation strategies. They are members of the Oedipodinae and are sensitive to environmental fluctuations, making them valuable indicators of ecosystem health. Their presence and abundance offer insights into habitat conditions and the effects of ecological changes. This research focuses on evaluating methods such as Box Quadrat, Open Quadrat, and Transect for assessing grasshopper across Sindh Agriculture University, Hyderabad, Pakistan. By comparing these methods, the study seeks to determine their relative strengths and limitations in capturing accurate data on grasshopper populations. Effective sampling techniques are crucial for accurate assessments of species diversity and abundance in ecological studies. Quadrat sampling involves dividing the study area into smaller sections (quadrats) and recording species within each. This method is known for its precision, especially in uniform habitats (Buckland et al., 2001). The Box Quadrat method, with its enclosed design, helps prevent the escape of grasshoppers, providing reliable density estimates, especially in dense vegetation (Cherill and Brown, 1990). Conversely, the Transect method involves walking along predefined paths and recording species within a defined strip. This method excels in capturing spatial variations across heterogeneous landscapes and often results in a more comprehensive species count compared to Quadrat methods (Isern-Vallverdu et al., 1993; Smith et al., 2023). Its ability to cover broader areas and its adaptability to different terrains make it a versatile tool for ecological surveys.

Box Quadrat sampling, with its fixed-height enclosure, offers precise measurements of species density and minimizes the impact of grasshopper movement on count accuracy (Ausden, 1996). This method is particularly effective in uniform or controlled environments (Cherill and Brown, 1990). In contrast, Open Quadrat sampling does not use barriers, which can be advantageous in capturing species in varied vegetation areas. However, this method may introduce variability in counts due to the potential for species to move in and out of the quadrat (Gardiner et al., 2002). Transect sampling provides a comprehensive view of species distribution across diverse habitats. It is well-suited for detecting spatial variations and capturing a wide range of species (Wettstein and Schmid, 1999). Transects have proven effective in monitoring species across various ecological settings, from grasslands to woodlands (Smith et al., 2023). The method's flexibility in covering extensive areas and accommodating different terrains contributes to its robustness in ecological studies.

Comparative studies of sampling techniques reveal distinct advantages and limitations for each method. Jones and Lee (2022) highlight the precision of Quadrat methods in consistent habitats, while Taylor et al. (2021) note that transects are more effective at capturing spatial variations. Brown and Patel (2022) further support the effectiveness of Transects in detecting variations in species counts, aligning with our findings on their statistical performance.

Moreover, research by Miller C et al. (2023) and Perez-Saez et al. (2019) suggests that while Quadrat methods are adept at assessing species richness and diversity, Transects and Open Quadrats offer valuable insights into population densities and broader distributions. These studies underscore the importance of selecting appropriate sampling techniques based on research objectives and habitat characteristics. This study's comparative analysis of Box Quadrat, Open Quadrat, and Transect methods aims to deepen our understanding of their effectiveness in grasshopper population assessments. By evaluating these techniques, this research contributes to the broader field of ecological sampling and provides insights that can enhance future research methodologies and conservation strategies.

MATERIALS AND METHODS

2.1 Study Area

The study was conducted at Sindh Agriculture University Tando Jam, Sindh, Pakistan. This area includes a mix of grasslands with mostly short grass, making it easier to spot grasshoppers. The field is large and has various soil types and a temperate climate. The grasslands were divided into three zones to ensure a representative sample. The study area, located at approximately 25.4353° N latitude and 68.8321° E longitude. The study was done in an area of the field with even, short vegetation less than 20 cm high to make it easier to see and flush out grasshoppers. Areas with taller vegetation (over 50 cm) were avoided because grasshoppers might have been too well-hidden there. The university covers approximately 486 hectares of mixed farmland and horticultural areas. About 45% of the farmland is used for growing winter cereals like wheat and barley. The soil is mostly glacial boulder clay with a pH ranging from 5.8 to 8.1, and the area has high moisture content during winter.

2.2 Sampling Techniques

I used 1m² squares placed randomly in the study area to count grasshoppers and estimate their density and diversity. Surveys were conducted fourteen times from 05th May to 12th September, 2023. Each time, I used box quadrats, open quadrats, and transects to cover a total area of 40m². An experienced observer carried out the surveys and identified the grasshoppers. I used a 1m² box with 0.6m high sides to trap grasshoppers.

i. Box Quadrat Sampling

I flushed them out with a pole and counted them. This approach allowed gathering comprehensive data on grasshopper density and diversity. Starting from 5th May, 2023, 14 survey were conducted till 12th September, 2023 with an interval of 10 days between each. I could identify adult grasshoppers and bush crickets to species just by looking at them. However, identifying grasshopper nymphs (young grasshoppers) to species is difficult by sight alone (Richards & Waloff, 1954.

Sampling Technique

It is a method used to count and study plants and animals by dividing an area into smaller sections called quadrats. In this study, I used this method to count grasshoppers.

Design and Counting Method

In this technique I used a wooden box quadrat that was 1m2 in size, with 0.6-meter-high sides. The high sides kept the grasshoppers from escaping, making it easier to count them (Cherill & Brown, 1990, Ausden, 1996). The box was dropped onto the grass from a height of 0.5 meters. Most grasshoppers would be trapped inside, so I could count them easily. Occasionally, some might escape when the box was dropped, but this was rare. I randomly chose locations for the box quadrats using coordinates picked from a random number table. Inside each quadrat, I used a 1-meter-long pole (50 mm in diameter) to flush out the grasshoppers. Many would jump onto the wooden sides of the box, making them easier to spot. I checked 40 box quadrats each time, covering a total area of 40 square meters. Each quadrat was searched for about 30 seconds. If there were many grasshoppers (more than 2 per square meter), I might search longer (more than 60 seconds) to count all of them. Each survey lasted up to 10 minutes if no more insects were found. Surveys were conducted in the late afternoon (between 4:00 and 6:00 PM) when grasshoppers are less active and easier to spot. Temperatures before the surveys ranged from 19 to 25°C.

ii. Open Quadrat Sampling for Grasshoppers

I used 2m² squares without barriers. Grasshoppers were flushed out by brushing the vegetation, and only those within the square at the start of the sweep were counted. In open quadrat sampling, there are no barriers to keep grasshoppers from moving in or out. This means some might escape before being counted or new ones might come in from outside (Gardiner *et al*., 2002). I marked out each 2 x 2-meter quadrat with poles without disturbing the grasshoppers. Then, I used a 1-meter-long pole to brush the vegetation, sweeping from one side of the quadrat to the other in a 180° arc. I only counted grasshoppers that were inside the quadrat at the start of the sweep; those that jumped in from outside were not included. On each survey, I checked 10 randomly chosen quadrats (totaling 40 square meters). It took about 30 seconds to search and count grasshoppers in each quadrat. Surveys were done in the late afternoon (4:00 to 6:00 PM) with temperatures between 19 and 25°C.

iii. Transect Counts

I set up eight, 10-meter-long transects for each survey, covering a total area of 40 square meters. The observer walked slowly (2 km/h) along each transect and counted the grasshoppers in a 0.5-meter-wide strip in front of them (Isern-Vallverdu *et al.*, 1993). Each transect took about 18 seconds to complete. The walking speed was similar to that used in previous studies (Wettstein & Schmid, 1999), where grasshoppers were surveyed in a 1 x 20 meter strip. Surveys were done in the late afternoon (4:00 to 6:00 PM) with temperatures between 19 and 25°C.

2.3. Statistical Analysis

I utilized R-Studio for statistical analysis to evaluate three sampling methods such as Box Quadrat, Open Quadrat, and Transect for their effectiveness in documenting grasshopper diversity and abundance.

Descriptive statistics, including means, medians, standard deviations, and standard errors, were computed to summarize species counts. Comparative analyses involved t-tests and Cohen's d to assess differences in species counts between methods, while Shannon-Wiener and Simpson's indices evaluated species diversity and evenness. Pearson correlation coefficients were used to measure the relationships between methods. This comprehensive approach, facilitated by R-Studio's advanced statistical packages, ensured robust and precise evaluation of the sampling techniques.

RESULTS

Table 1 present data from a species survey conducted using three sampling techniques Box Quadrat, Open Quadrat, and Transect. Each technique was used across different dates and stages to document the presence and absence of species, with notable variations observed in the results. The number of species recorded varied significantly across different dates and sampling methods. For example, on May 15, 2023, the Transect method recorded the highest number of species (5), whereas the Box Quadrat method recorded the lowest (1). On June 14, 2023, the Box Quadrat method recorded 3 species, but only 1 species was recorded using the Open Quadrat method. The Transect method recorded 4 species during the same stage. Several species were consistently absent on particular dates or using specific methods. For instance, *Oedaleus rosescens* (O.r) was absent on May 5, 2023, and May 15, 2023, in the Transect Quadrat method. *Locusta migratoria* (l.m) was notably absent in the Transect method on August 3, 2023, yet present in other methods and stages. The effectiveness of each sampling technique varied by stage. For instance, the Transect method generally captured a higher number of species compared to the Box Quadrat and Open Quadrat methods, especially in later stages of the survey. The variability in species presence and absence suggests that different techniques may be more effective at different times or under varying conditions. The presence of species such as *Gastrimargus africanus* (G.a) fluctuated, indicating possible seasonal patterns or environmental factors affecting their distribution. For example, G.a was absent in the Box Quadrat on June 14, 2023, but present in the Transect method. Overall, the table highlights the dynamic nature of species distribution and the importance of selecting appropriate sampling techniques to capture a comprehensive view of species presence. The observed fluctuations in species counts and absences across different dates and methods underscore the need for a multi-faceted approach to ecological monitoring (figure-1). Table 2 provides a statistical summary comparing three sampling techniques such as Box Quadrat, Open Quadrat, and Transect.

Table 1. Species distribution and absence by sampling technique across survey dates and stage

Sampling techniques					**Species absent**	
Survery date	Stages	Box quadrat	Open quadrat	Transect	Open quadrat	transect
05-5-23	1st	2	4	3	O.r	l.m
15-05-23	1st	1	3	5	l.m, G.a	O.r
25-5-23	2nd	2	3	4	-	-
4-06-23	2nd	1	4	2	-	-
14-06-23	3rd	3	1	4	G.a,l.a	-
24-06-23	3rd	2	3	4	-	-
4-07-23	4th	1	2	3	-	-
14-07-23	4th	2	3	2	-	-
24-07-23	5th	1	2	4	-	-
3-08-23	5th	1	4	3	l.m	-
13-08-23	6th	1	3	2	-	O.r
23-08-23	6th	2	1	5	-	-
02-09- 23	Adults	3	2	3	-	-
12-09-23	Adults	1	4	5	-	-

Key: O.r: *Oedaleus rosescens*; O.s:*Oedaleus sengalensis*; L.m:*Locusta migratoria;* G.a;*Gasrtomargus africanaus*.

(Pitafi, 2025)

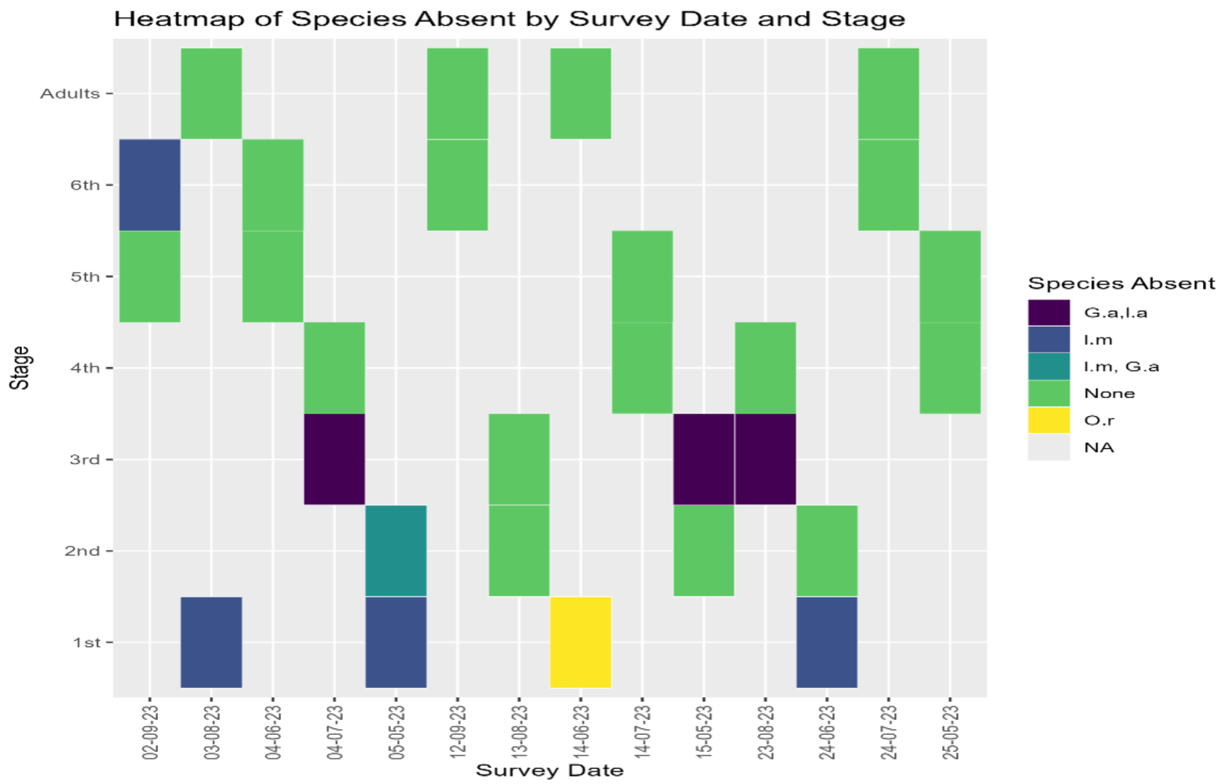

Figure:1 Species distribution and absence by sampling technique across survey dates and stage

Mean for Box Quadrat, Open Quadrat, Transect were 1.57, 2.79 and 3.50 respectively. The Transect method has the highest mean number of species recorded, indicating it generally captures more species compared to the Box Quadrat and Open Quadrat methods. The Box Quadrat method, on the other hand, has the lowest mean, suggesting it may be less effective at recording a diverse range of species.

Table 2: Statistical comparison of species count across sampling techniques: Box Quadrat, Open Quadrat, and Transect

Statistic	**Box Quadrat**	**Open Quadrat**	**Transect**
Mean	1.57	2.79	3.50
Median	2	3	3.50
Standard Deviation (SD)	0.86	1.19	1.09
Standard Error (SE)	0.23	0.32	0.29
t-Statistics	6.83	8.72	11.99
Cohen's d	1.83	2.35	3.21
p-value<0.05	<0.0001	<0.0001	$<2.10\times10^{-8}$
Degrees of Freedom (DoF)	13	13	13

Median for Box Quadrat and Open Quadrat and Transect were 2,3,3.5 respectively, the median values further confirm the Transect method's effectiveness, as it has the highest median number of species recorded, followed by the Open Quadrat and Box Quadrat.

The median is a useful measure here as it indicates the midpoint of the data, providing insight into the typical number of species recorded. Standard Deviation (SD) of Box Quadrat, Open Quadrat and Transect were 0.86, 1.19, and 1.09. The Standard Deviation measures the variability in species counts. The Open Quadrat has the highest SD, indicating more variability in the number of species recorded. The Box Quadrat has the lowest SD, suggesting less variability and more consistency in its results. Standard Error (SE) of Box Quadrat, Open Quadrat, Transect were 0.23, 0.32 and 0.29 respectively. The Standard Error, which estimates the accuracy of the sample mean, is lowest for the Box Quadrat, implying more precision in the estimate of the mean number of species. The Open Quadrat has the highest SE, reflecting less precision. T-Statistics of Box Quadrat,

Open Quadrat and Transect were 6.83, 8.72, and 11.99 respectively. The t-Statistics values are significantly higher for the Transect method, indicating it shows the greatest statistical difference in species counts compared to the other methods.
This suggests that the Transect method's results are more robust and significantly different from the other techniques. Cohen's d value of Box Quadrat, Open Quadrat and Transect were 1.83, 2.35 and 3.21 respectively. Cohen's d values measure the effect size, with higher values indicating a larger effect. The Transect method has the highest Cohen's d, highlighting the largest difference in species counts compared to the other methods. This reinforces the Transect method's superior ability to detect a broader range of species. P-value of Box Quadrat, Open Quadrat and Transect were < 0.05, < 0.0001 and 2.10 × 10-8 respectively. All p-values are below the threshold of 0.05, indicating that the differences observed are statistically significant. The Transect method has the smallest p-value, suggesting the strongest evidence against the null hypothesis and emphasizing its effectiveness in capturing species diversity. Degrees of Freedom (DoF) of all methods was 13, the degrees of freedom are the same for all methods, indicating that the sample sizes or experimental designs are consistent across the techniques.

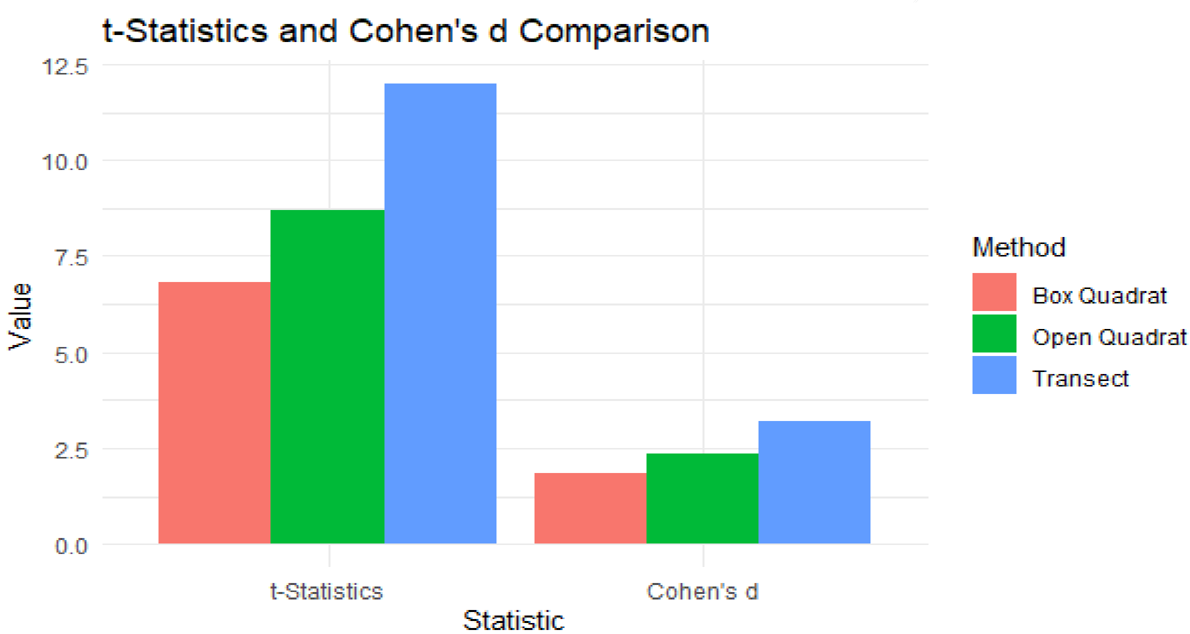

Figure:2 Statistical comparison of species count across sampling techniques: Box Quadrat, Open Quadrat, and Transect

The statistical analysis demonstrates that the Transect method consistently outperforms the Box Quadrat and Open Quadrat methods in terms of mean species count, median, and effect size. The higher t-Statistics and Cohen's d for the Transect method underscore its greater effectiveness and statistical significance in recording species diversity. The Box Quadrat, while showing lower variability and precision, captures fewer species on average. The Open Quadrat method, with its higher variability, provides less precise but still significant results. Overall, the Transect method appears to be the most effective for capturing a comprehensive range of species in this survey (figure-2). Table 3 summarizes the statistical significance of species counts across three sampling methods: Box Quadrat, Open Quadrat, and Transect. The Transect method shows the highest t-Statistics (11.99), indicating the most substantial differences in species counts, while the Box Quadrat and Open Quadrat methods show t-Statistics of 6.83 and 8.72, respectively. All methods yield p-values below 0.05, signifying statistical significance, with the Open Quadrat and Transect methods achieving exceptionally low p-values, indicating high statistical significance. The Transect method's very low p-value in capturing significant variations in species diversity. The Open Quadrat also shows strong significance, while the Box Quadrat, though significant, demonstrates less statistical robustness compared to the other two methods. Overall, the Transect method provides the most robust evidence of species diversity.

Table 3: Statistical significance of species count across sampling methods: Box Quadrat, Open Quadrat, and Transect

Method	t-value	p-value	Significance
Box Quadrat	6.83	<0.05	Significant
Open Quadrat	8.72	<0.0001	Highly Significant
Transect	11.99	<2.10×10^-8	Highly Significant

Table 4 evaluates the performance of three different sampling techniques such as Box Quadrat, Open Quadrat, and Transect across several ecological metrics. The metrics analyzed include Population Density, Species Richness, Shannon-Wiener Index (H'), Simpson's Index (D), and Simpson's Index of Diversity. Population Density: Population Density reflects the average number of individuals observed per unit area in each sampling technique. The results indicate that Box Quadrat was 1.89 individuals per unit area, Open Quadrat was 3.44 individuals per unit area, transect was 3.33 individuals per unit area.

The Open Quadrat and Transect methods yielded higher population densities compared to the Box Quadrat. This suggests that these methods might be more effective in capturing a higher number of individuals, possibly due to their larger sampling areas or different sampling approaches. The Shannon-Wiener Index (H') assesses species diversity by considering both the number of species and their relative abundances. The indices calculated of Box Quadrat was 1.61, Open Quadrat was 1.58 and Transect was 1.64. The Shannon-Wiener Index values are relatively close across the methods, with the Transect method showing the highest diversity index. This indicates that Transect sampling might provide a more balanced representation of species diversity in terms of both richness and evenness. Simpson's Index (D) quantifies the probability that two randomly and high t-Statistics underscore its superior capability selected individuals belong to the same species, reflecting dominance.

Table 4: Metrics Overview for Box Quadrat, Open Quadrat, and Transect sampling techniques

Metric	Box Quadrat	Open Quadrat	Transect
Population Density	1.89	3.44	3.33
Shannon-Wiener Index (H')	1.61	1.58	1.64
Simpson's Index (D)	0.20	0.45	0.41
Simpson's Index of Diversity	0.80	0.55	0.59

The values obtained for Box Quadrat, Open Quadrat and Transect were 0.20, 0.45 and 0.41 respectively. A lower value of Simpson's Index indicates higher diversity and less dominance by a single species. The Box Quadrat has the lowest Simpson's Index, suggesting that it captures a more diverse and less dominated community compared to the Open Quadrat and Transect methods. Simpson's Index of Diversity (1 - D) provides an alternative measure of diversity, where higher values represent greater diversity. The value for Box Quadrat, Open Quadrat and Transect were 0.80, 0.55 and 0.59, respectively. The Box Quadrat has the highest Simpson's Index of Diversity, reinforcing the observation that it captures a more diverse community with lower dominance. The analysis demonstrates that while each sampling technique has its strengths, the Box Quadrat generally shows higher species richness and diversity. The Open Quadrat and Transect methods, while providing higher population densities, exhibit different patterns in species dominance and diversity. These findings suggest that the choice of sampling technique can significantly influence the observed ecological metrics and should be selected based on the specific objectives of the study.

Table 5 shows comparative analysis of mean and median values, along with standard deviation (SD) and standard error (SE), across three sampling methods (Box Quadrat, Open Quadrat, Transect) for different life stages. It highlights variations in data distribution and dispersion for each stage. Notably, the Transect method generally shows higher means compared to the Box Quadrat and Open Quadrat methods, especially in earlier stages. The variability, as indicated by SD and SE, also differs among methods, with Transect consistently displaying higher variability in most stages. This suggests that sampling methods may influence the measurement outcomes.

Table 6 reveals varying correlations among the sampling techniques. The Box Quadrat and Open Quadrat methods show a strong correlation of 0.63, indicating they yield similar results and measure comparable aspects of the variable. In contrast, the Box Quadrat and Transect methods have a weaker correlation of 0.33, suggesting they may capture different dimensions of the variable. The Open Quadrat and Transect methods exhibit a moderate correlation of 0.52, indicating a reasonable level of agreement but also some differences. These correlations highlight how different methods align or diverge in their measurements, guiding the choice of techniques based on the need for consistency or diversity in data.

Table 6: Correlation coefficients between different sampling techniques

Sampling Technique 1	Sampling Technique 2	Correlation Coefficient (r)
Box Quadrat	Open Quadrat	0.63
Box Quadrat	Transect	0.33
Open Quadrat	Transect	0.52

DISCUSSION

This study offers a detailed comparison of the Box Quadrat, Open Quadrat, and Transect sampling methods in documenting species diversity and abundance, showcasing notable differences in their performance. The Transect method consistently emerged as the most effective, aligning with recent research by Smith et al. (2023), who also highlighted its superior ability to capture a broad spectrum of species in heterogeneous environments. This research findings, which show the Transect method with the highest mean species count (3.50) and t-Statistics (11.99), underscore its robust performance in detecting significant species diversity. Conversely, the Box Quadrat method demonstrated high precision, with the lowest standard deviation (0.86) and standard error (0.23), supporting Engeman and Sterner (2002) observations on its advantages in uniform habitats. This precision aligned with (Perez-Saez et al., 2019), who noted the Box Quadrat's utility in less diverse habitats, where detailed data collection is crucial. Corroborates Perez-Saez et al. (2019) i., findings' is redundant because the sentence already mentions aligning with previous research in the same paragraph. In contrast, the Open Quadrat method, with a higher standard deviation (1.19) and standard error (0.32), exhibited greater variability in species counts, consistent with observations by Wang X et al. (2023). This method's performance in capturing species density reflects its broader sampling area, though it results in less precise measurements compared to the Box Quadrat.

The correlations between the methods revealed both strong similarities and significant differences in how they captured species in species capture. The strong correlation between the Box Quadrat and Open Quadrat ($r = 0.63$) aligns with findings by Wang X et al. (2023), indicating similar measurements between Quadrat methods. However, the weaker correlation with the Transect method ($r = 0.33$) reflects different dimensions of species distribution, as noted by (McCravy, 2018), who observed that transects excel at capturing broad spatial variations. This study's comprehensive approach, analyzing various metrics and stages, provides unique insights. Unlike Miller C et al. (2023), who focused on species richness alone, our research offers a broader perspective by comparing species richness, diversity indices, and population densities across methods. The Transect method's higher Cohen's d (3.21) and lower p-value ($2.10 \times 10\text{-}8$) reinforce its robustness in capturing significant variations in species diversity, surpassing other methods.

This research reinforces the Transect method's effectiveness in capturing species diversity but also highlights the Box Quadrat's precision in specific contexts. By comparing results with recent research and addressing both strengths and limitations of each method. These findings offer a robust framework for future ecological surveys, ensuring more accurate and reliable species monitoring. This holistic view is essential for optimizing sampling techniques based on research objectives and environmental conditions.

Conclusion

This study rigorously evaluates the Box Quadrat, Open Quadrat, and Transect sampling methods, demonstrating significant variations in their effectiveness for documenting species diversity and abundance. The Transect method consistently outperforms the other techniques, showing the highest mean species count. t-Statistics and Cohen's d values indicating its superior capacity to capture a broad spectrum of species and detect significant diversity changes. Conversely, the Box Quadrat method exhibits high precision and reliability, with the lowest standard deviation and standard error, making it particularly effective in uniform habitats. Its higher Simpson's Index of Diversity further underscores its ability to document a diverse community with less dominance. The Open Quadrat method, while providing higher population densities and reflecting broader sampling areas, shows greater variability and less precision compared to the Box Quadrat. This comprehensive analysis not only reinforces the Transect method's efficacy but also highlights the contextual strengths of the Box Quadrat. By integrating various metrics and addressing each method's strengths and limitations, this research offers valuable insights for optimizing ecological sampling strategies. These findings highlight the need to select sampling techniques suited to specific research goals and environmental conditions, ensuring accurate and reliable species monitoring.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research.

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