

Bacterial Succession and Carcass Specific Microbial Dynamics during Vertebrate Decomposition in a Tropical Environment

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ABSTRACT

Comparisons of multiple species and tropical environments are poorly represented in necrobiome studies, and microbial succession is a key aspect of vertebrate decomposition. The literature has not yet provided any data on the temporal and carcass specific pattern of culturable bacteria associated with five vertebrate taxa (fish, bird, rat, toad and lizard) under natural tropical conditions in Southern Nigeria. Ten carcasses from each vertebrate (n=10) were sampled sequentially throughout the process of decomposition with only one carcass analysed each day. Serial dilution, triplicate replicate plating and conventional biochemical identification of tissue samples were performed. No differences in recovery of the four predominant bacterial species (*Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus licheniformis* and *Escherichia coli*) were found by carcass type. *P. aeruginosa* dominated early decomposition (Days 1-3) followed by an increase in *B. licheniformis* and *E.coli* during mid to late decomposition, as aerobic to increasingly anoxic conditions occurred. Community structure was affected by carcass type, such that fish represented the highest proportion of *Pseudomonas*, birds had increased levels of *Staphylococcus* and lizards had increased proportions of *Bacillus*. Notably, the toad cadaver showed the lowest proportion of the abundant groups and the highest average number of bacteria per day among the samples showing the proliferation of less abundant groups of bacteria in its moist mucous surfaces. The results showed the bacterial succession is stage-dependent and significantly affected by the carcass identity in a tropical environment. Despite its cultural and exploratory nature, the study was also quantitative, and yielded replicated results of decomposition related microbial patterns in a range of vertebrate substrates.

KEYWORDS: Bacterial succession, Decomposition, Necrobiome, Forensic Microbiology

1.0 Introduction

The decomposition of the vertebrate remains is a very complex ecological process, with the participation of microorganisms and invertebrates, scavengers and environmental factors, and which will contribute to the recycling of organic matter and nutrients within ecosystem (McIntyre et al., 2026). Microorganisms, especially bacteria, are central to this process, as their biochemical transformation processes are crucial for regulating tissue degradation and the release of carbon, nitrogen and other nutrients to the surrounding environment (DeBruyn et al., 2025; Pecha let al.,

2013). The microbial community from the host, soil and external sources will interact and constitute a dynamic community called a necrobiome during the process of decomposition (Harrison et al., 2020). The microbial succession of bacterial communities involved in carrion is a predictable temporal change in abundance and composition. The main driver of these changes is decomposition stage, which affects the physicochemical conditions, in this case the oxygen, moisture and nutrient levels (Mclyntre et al., 2026; DeBruyn et al., 2025). The early stages of decomposition tend to be dominated by facultative and aerobic bacteria, whereas the later decomposition phases are dominated by anaerobic bacteria under hypoxic conditions created by the intense microbial metabolism (DeBruyn et al., 2025). Research has shown that these successional trends can be used to gain insight into ecosystem function and have proven useful in a forensic setting (Cobaugh et al., 2015) but their usefulness is dependent on region and environmental conditions. Although the field of necrobiome studies is developing, there are still many gaps. There are numerous studies that have been carried out in temperate ecosystems and/or in controlled environments, which are less applicable to those environments than tropical ecosystems, where microbial turnover and decomposition rates are faster due to high temperatures and humidity (Harrison et al., 2020; Howard et al., 2010). In addition, most of the literature focussed on single species models (e.g. pig or cattle), limiting the understanding of the influence of species-specific physiological traits on microbial trajectories (Dangerfield et al., 2020). Variable composition of tissues, integument structure, and moisture levels, along with variations in the endogenous microbial community, across vertebrate species may affect the pattern of colonisation, decomposition rates, and relative abundance of the major groups of bacteria (Zhou et al., 2021; Wallace et al., 2021). Comparative studies under the same environment for simultaneously exposed carcass types are in particular lacking, but these are crucial for discovering generalizations about ecological patterns and substrate-specific deviations from bacterial succession.

Finally, there is the influence of carcass type on the development of microbial communities, which has been little studied (Carter et al., 2015; Dangerfield et al., 2020); many studies of microcosms in controlled or semi-controlled experimental settings, such as laboratory-based or limited field situations, have been conducted. These methods can isolate specific variables but can't necessarily capture the complexity of natural ecosystems with variable environmental conditions and multiple interactions among organisms. It is thus necessary to carry out studies in natural outdoor conditions to gain insights into the microbial succession in ecologically meaningful environments.

A second constraint of existing research is the focus on molecular techniques that are very powerful but less useful for the study of culture based assessment of microbial communities, although these methods are still useful for quantifying viable bacterial communities and understanding the functional aspects of microbial succession and are used in relatively few longitudinal studies in natural environments. Another aspect, the understanding of microbial ecological dynamics is historically underrepresented in carrion research because the

decomposition studies have focused mainly on macrofaunal components, especially insects. More recent studies such as those of Holt et al. (2026) on interactions between insects and microbes and their role in decomposition work have emphasized the importance of interactions between microbes and invertebrates in influencing the decomposition process, but the contribution and dynamics of the bacterial components are less well understood.

In view of these shortcomings, the present study focused on the limitations and therefore investigated the temporal dynamics and carcass specific variation of culturable bacteria from decomposing vertebrate carcass under natural outdoor conditions in Southern Nigeria. This study offers quantitative, culture-based information on bacterial succession over time and how the identity of the carcass influences microbial community structure in a humid tropical environment using daily sampling on independently replicated carcasses.

2.0. Materials and Methods

The study was carried out in a humid tropical condition in Benin City, Southern Nigeria that had mean temperature of 27-32°C and relative humidity consistently high (slightly above 70%). These are environmental factors that are known to affect the dynamics of microbial proliferation and decomposition. The experimental site was an outdoor site located in the faculty of life sciences, University of Benin, where the carcasses were left to naturally, and not artificially, develop and undergo exposure to environmental conditions, thus allowing an ecologically realistic setting for the development of necrobiome succession.

2.1. Experimental Design and Carcass Exposure

Five orders of vertebrates, ranging in terms of ecological and anatomical characteristics, were selected: fish, bird, rat, toad and lizard. This selection allowed a comparative evaluation of the potential of the microbial succession and decomposition dynamics to be influenced by tissue composition, moisture and integument structure. A total of 10 (n=10) carcasses of each kind of vertebrates were collected from approved sources that were recently killed. Carcasses were utilized sequentially, one per day, so that each day's carcass was a new biological sample and not a replicated sample of the same carcass. The fresh specimens were retrieved and deployed right away to represent natural post mortem environments. Carcasses were all left on the soil surface and covered with ventilated, wooden mesh cages spaced 2m apart to exclude vertebrate scavengers and to provide unrestricted access by insects, airborne micro-organisms and environmental exposure. The carcasses were exposed at the same time to standardize the decomposition time and for the easy comparison of the pattern of bacterial colonization of the different types of carcass. This was a design that allowed temporal resolution of microbial succession while providing replication between decomposition days.

2.2. Sampling Procedure

The sampling was done once a day during the decomposition period usually at around 10:00 am to minimize the diurnal variability and achieve a temporal consistency. One carcass was sampled each day for each vertebrate species until there were 10 carcass types with advanced stages, and these could not be sampled. All samples were aseptically collected from decomposing tissues of each carcass using sterile scalpels and forceps and put into sterile containers were then transported on ice and processed for microbiological analysis within an hour of sample collection. Steps were taken to avoid cross contamination between samples. The time to decomposition was 4-9 days (depending on type of carcass) and was similar to that normally observed in tropical climates and at hot water temperatures.

2.3. Microbiological Analysis

Tissue sample (1g) was homogenized in 9ml of sterile phosphate buffered saline and serially diluted (10⁻¹-10⁻⁶). Each dilution was plated in triplicate so as to ensure reproducibility. Aliquots (0.1ml) were spread plated onto Nutrient, MacConkey, Mannitol salt and Centrimide agar. Inoculated plates were incubated for 24-48h under suitable conditions in the laboratory for the multiplication of bacteria. Colony forming units(CFU/mL) have been counted after incubation and the average of the triplets has been calculated. This culture dependent method allowed for dominant viable bacterial populations to be quantified and helped to generate high resolution temporal succession patterns that were matched with previously decomposition studies (Cobaugh et al., 2015; Suet et al., 2025).

2.4. Determination of the Bacterial Isolates.

Bacterial isolates were identified using standard biochemical test (Gram staining, oxidase, catalase, citrate utilisation, coagulase, TSI reactions) and morphological characteristics, which include pigmentation, shape and appearance. Confirmatory identification was done according to Bergey's Manual of protocols. A number of taxa were identified with 4 of them being consistently dominant. The quantitative succession analysis was based on these dominant species.

2.5. Quality Assurance and Quality Control(QA/QC)

Thorough quality assurance / quality control (QA/QC) measures were taken to assure data reliability. All Instruments were flame sterilised or disinfected with 70% ethanol between carcasses and sterility Controls: Uninoculated agar plates were incubated daily to check for contamination.

2.6. Replicates Plating: All serial dilutions were done in triplicate to account for technique variability

2.7. Media Verification: Media for each batch was tested for sterility and performance before use.

2.8. Routine Calibration of Instruments: Pipettes, incubators and weighing balances were routinely calibrated to the standards established in the laboratory.

2.9. Sample Independence: Each day's carcass was a separate biological replicate, not introducing any resampling bias.

Such measures provided methodological uniformity and reduced contamination and/or measurement errors.

2.10. Data processing and Relative Abundance Calculations.

CFU counts were made on a species type basis, and for each bacterial species, the Relative abundance (%) was determined by dividing the count of that species by the total count of all species as in equation below:

$$\text{Relative abundance} = \frac{\text{CFU of species}}{\text{Total CFU of all species}} \times 100$$

Daily values were used to generate Species-specific succession curves(Figure 1), A decomposition-stage heatmap(Figure 2), Carcass specific mean abundance profiles (Figure 3) and a stacked compositional profiles (Figure 4).

2.11. Data Analysis

Data were analysed using Excel and cross validated with Python based scripts. Mean $\pm$ SD values for each species across carcasses (Table 1) were computed for summary comparison.

Because one carcass was used per day for each vertebrate, statistical analysis reflect independent daily replicates rather than repeated measures. The analysis are therefore exploratory and intended to characterize trends rather than provide confirmatory inferential conclusions.

2.12. Ethical Considerations

All carcasses were obtained and handled in accordance with institutional and national guidelines. The study did not involve experiments on live animals.

3.0. RESULTS

Bacterial colonisation of decomposition of the five vertebrate carcasses showed clear stage dependent succession patterns with both temporal and carcass specific differences evident across decomposition period. Four dominant taxa *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus licheniformis*, and *Escherichia coli*, were consistently recovered across vertebrate groups, although their relative abundances varied across days and carcass type.

Table 1. Summary dominant bacterial taxa across carcass types and decomposition days

Species	Fish		Bird		Rat		Toad		Lizard	
<i>Pseudomonas</i>	26.33	±	32.50	±	31.71	±	34.75	±	42.00	±
	17.14		18.39		19.62		16.62		30.24	
<i>Bacillus</i>	11.44	±	13.50	±	10.71		13.75 ±8.81		14.75	±
	7.38		10.45		±5.44				4.99	
<i>Staphylococcus</i>	4.44 ± 5.92		12.17 ± 5.81		7.14 ± 7.56		7.75 ± 5.50		11.50	±
									3.79	
<i>E. coli</i>	5.11 ± 5.11		7.00 ± 6.39		7.57 ± 7.79		3.00 ± 6.00		2.25 ± 4.50	

Temporal dynamics of bacterial succession.

In each of the carcass types bacterial succession was predictable, with '*Pseudomonas aeruginosa*' dominating the early decomposition interval (Day 1-3) (Figure 1). This early stage peak coincided with high aerobic activity, and with a quick consumption of readily available substrates that were released soon after death. Relative abundance of *Pseudomonas aeruginosa* decreased during decomposition, while the representation of *Bacillus licheniformis* (Days 4-6) and *E. coli* (Days 6-9) increased in abundance in the mid and late stages of decomposition (Figure 2). *Staphylococcus epidermidis* was less abundant in the early stages, but regularly decreased as the tissues became anoxic.

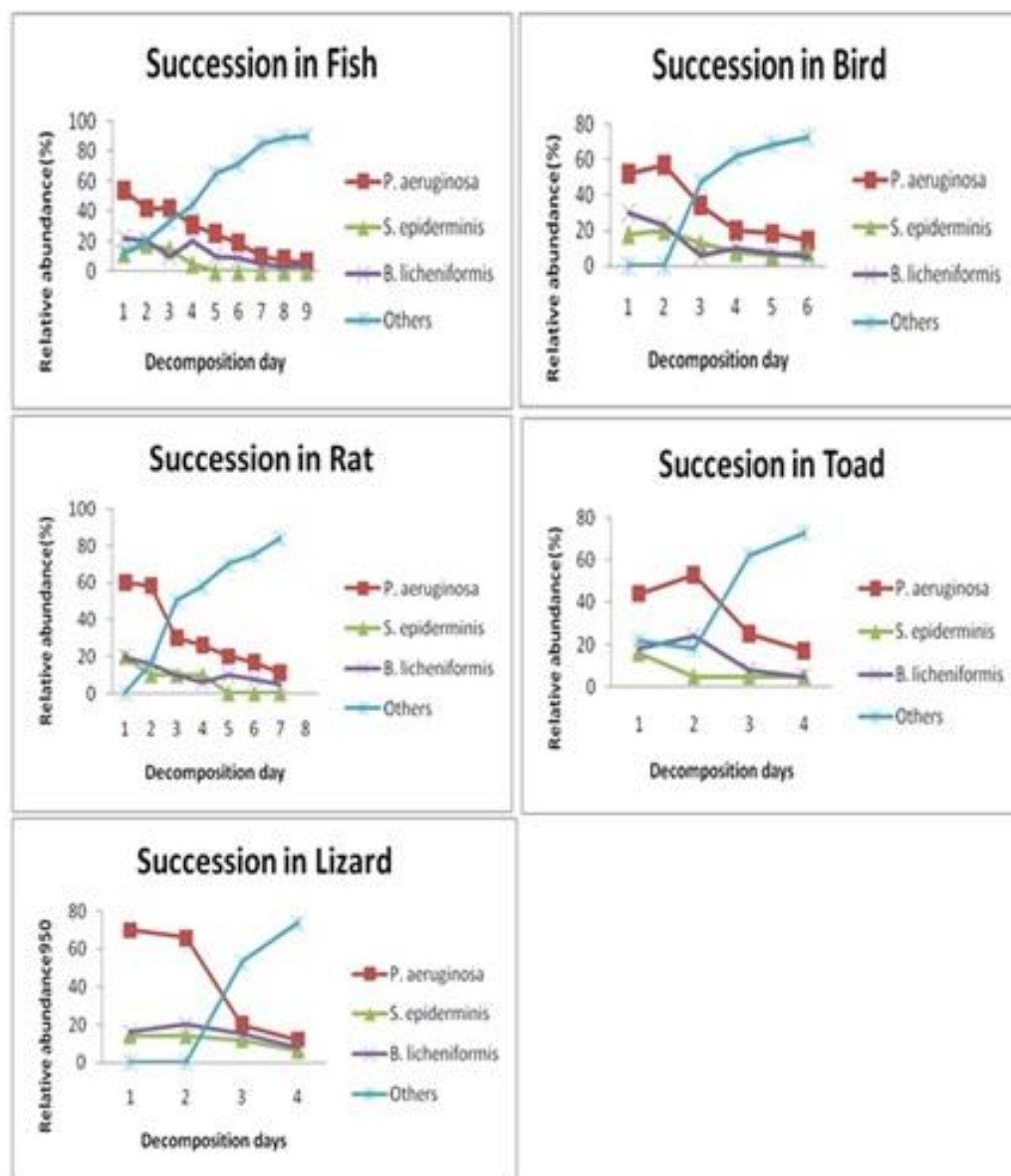


Figure 1. Temporal succession in the predominant bacterial taxa on carcasses of the different species. line plots of the daily relative abundance(%) of the predominant taxa of (A) Fish, (B) Bird, (C) Rat, (D) Toad and (E) Lizard carcasses. There is a unique but stage dependent succession pattern in each carcass with early colonization of opportunistic taxa followed by shifts to more specialized decomposers.

Day	<i>P. aeruginosa</i>	<i>B. licheniformis</i>	<i>S. epidermidis</i>	<i>E. coli</i>
1	54	21.2	16	0
2	55.2	20.6	13.4	0
3	30.2	9.8	11	1.8
4	21.2	9.8	6.8	9.4
5	12.6	4.4	1.2	5.8
6	10	4.4	1.6	7.6
7	4.2	2	0	5.4
8	1.6	0.6	0	2.4
9	1.2	0.8	0	0

Figure 2. Heat map of averaged bacterial abundance across decomposition days. Mean relative abundances (%) of the four dominant bacterial species were averaged across all carcasses for each sampling day. Warmer colours represent higher abundance. The heat maps reveals clear temporal transitions, beginning with *Pseudomonas* dominance in early decomposition and shifting towards *Bacillus* and *E. coli* in mid to late stages.

These stage associated shifts were observed across all vertebrate groups, indicating a robust successional trajectory under tropical field conditions.

Carcass Specific Patterns in Dominant Bacterial Taxa

Very different microbial profiles were seen among the five vertebrate carcass type (Figure 3). *Pseudomonas aeruginosa* was the most frequently found species in fish carcasses, and this species was found at highest relative abundance in a rich in moisture substrate. Proportionally high *Staphylococcus epidermidis* was found in bird carcass, which was assumed to be related to integument origin microdata. Comparatively balanced distribution of dominant taxa was found in the carcasses of rats whereas lizard carcasses had higher contribution of *Bacillus licheniformis* (Figure 4), which seems to colonise keratinised tissues..

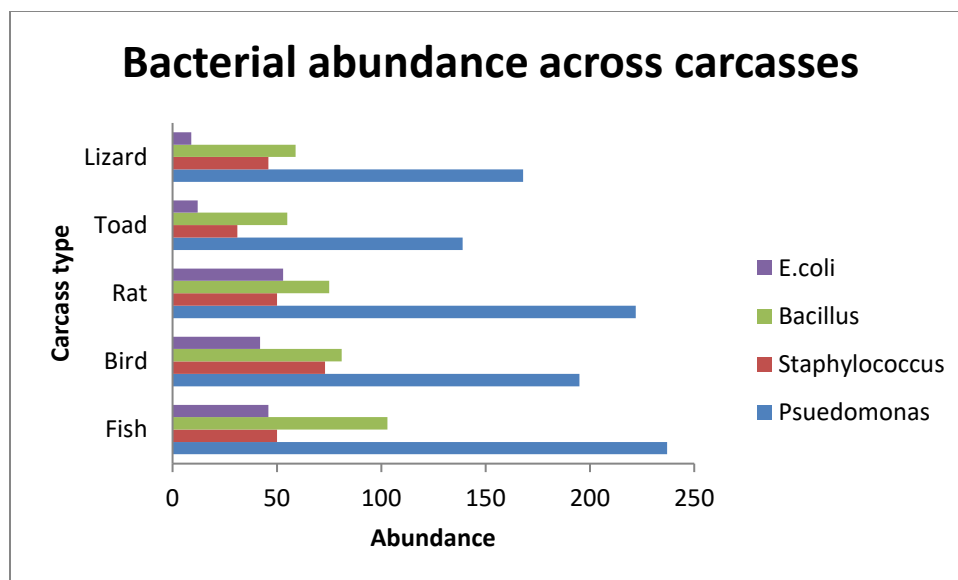


Figure 3. Relative abundance of key taxa on carcasses. The relative abundance (%) of *P. aeruginosa*, *B. licheniformis*, *S. epidermidis* and *E. coli* are shown in grouped bar charts from Fish, Bird, Rat, Toad and Lizard carcasses. The analysis of specific differences in microbial communities between the different carcass compartments demonstrates influence of tissue composition and inherent microbiota on the colonization process.

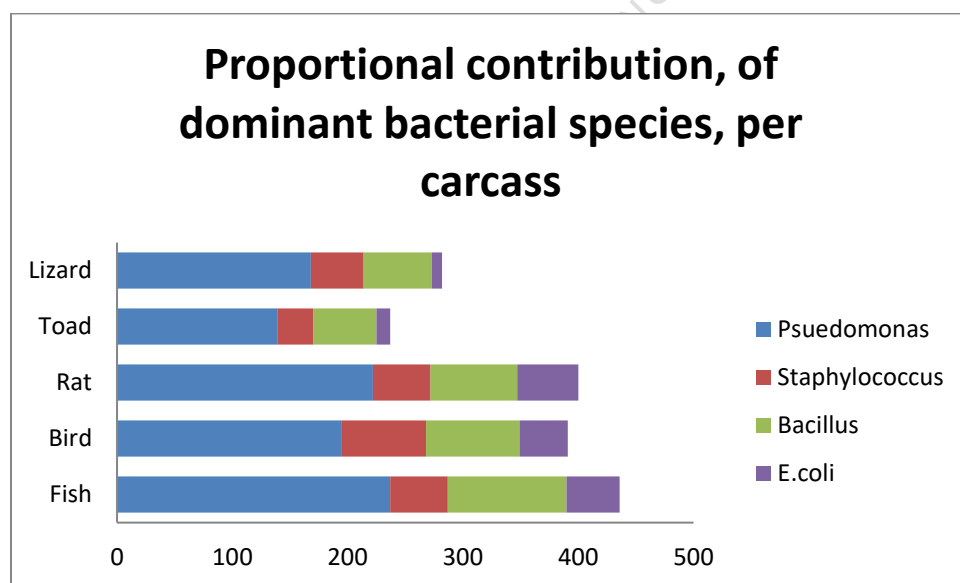


Figure 4. Proportional contribution of dominant bacterial taxa to each carcass. Stacked bar chart indicating the relative contribution of each bacterial species to the total bacterial community in each carcass type. Colours represents individual taxa. The figure demonstrates distinct compositional signatures for each carcass, with aquatic and terrestrial organisms exhibiting contrasting microbial profiles.

Total Bacterial Load Across Carcass type

Toad with the lowest mean daily relative abundance (Table 2) had the highest mean daily total bacteria load (Table 2). This pattern is indicative of the significant increase in non-dominant microbial populations found in the mucosal tissues of amphibians where it is possible to detect microbes that were present in the environment and transient in the mucosal tissue but were not included in the list of dominant microbes quantified. The total load from fish and lizard carcasses was observed to be instantaneous whereas that of the bird and rat carcasses were comparatively low daily loads.

Table 2: Total bacterial abundance across carcass types

Total Count	Bacteria	Total Count	Bacteria	Sampling Duration(Days)	Mean Daily Count
Fish		685		9	76.1
Bird		490		6	81.7
Rat		479		7	68.4
Toad		420		4	105.0
Lizard		334		4	83.5

Temporal structure of succession was observed as early-stage dominance of *Pseudomonas* and later increase in the levels of *Bacillus* and *E. coli*. The microbial composition was modulated by the carcass type, associated with differences in tissue chemistry, moisture and integument structure. The four predominant species were not found to represent a high proportion of the bacteria found but Toad carcasses had high absolute bacterial counts at lower relative abundance. The trends in time and substrate were supported by independent replicate carcass and triplicate plate counts.

4.0. Discussion

This study found that bacterial succession was observed and had a distinct temporal pattern with rapid early colonization of the pipe, followed by fluctuations in abundance. This is consistent with known models of necrobiomes, in which microbial communities experience a series of stage-dependent changes due to variations in available substrates, oxygen gradients, moisture and biochemical decomposition products (Carter et al., 2015; DeBruynet al., 2025). As shown in the succession curve in Figure 1a- e all the carcass showed a rapid increase in the number of bacteria, which in this case are largely opportunistic aerobes and anaerobes like *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus licheniformis*. This early microbial bloom may be due to the rapid release of intracellular nutrients after death. These taxa also grow rapidly after death because of the sudden release of fluids which are rich in nutrients and lack in immune regulation. This phenomenon is consistent, with previous necrobiome studies, which found a predominant trend of Proteobacteria, and Firmicutes early colonization, because of their ability to use simple, substrates and adapt to, oxygen changes (Liu et al., 2023; Dash & Das, 2020).

Bacterial growth was also more rapid and intense, however, than in temperate environments, where microbial growth is slower due to colder temperatures, which limit microbial activity (Harrison et al., 2020). In contrast, decreased early growth has also been reported under conditions of reduced moisture (desiccated toad carcasses) and in substrates that have antimicrobial defences (Crippen & Benbow, 2015), which are similar to the moderated trends in toad carcasses.

The intensity and direction of these initial peaks however, were different for carcasses types. The levels of bacteria were highest in fish carcass samples, reflecting the often fast decomposition that occurs in water and moist tissues. The increase in water content and decrease in structural resistance may have increased nutrient diffusion, allowing for more rapid microbial colonisation. Moreover, the relative softness of the tissue structure and low level of physical barriers in fish may also facilitate quicker penetration of microorganisms and colonization (Metcalf et al., 2016). The same trend of higher microbial growth at higher moisture conditions has been observed during aquatic and semi aquatic decomposition experiments (Benbow et al., 2015). The lower bacterial count in toad carcasses, on the other hand, indicate that the integuments and antimicrobial peptides of the toads reduced the invasion of microbes. It is well documented that amphibian skin produces antimicrobial peptides which prevent bacterial growth (Crippen & Benbow, 2015; Rollin-Smith, 2009). The differences illustrated in figures 3 and 4 highlight the role that intrinsic biological characteristic plays in the microbial dynamics during decomposition, which is underscored by this carcass dependent variation.

The high mean daily abundance of bacteria on lizard carcass, when compared to the decomposition period, indicated a rapid and intense colonization in a shorter time. This observation is consistent with the previous one that smaller, more compact organisms are more likely to decompose faster because they have more surface area to volume ratios to allow for deeper penetration by microorganisms and higher enzyme activity (Howard et al., 2010; Metcalf et al., 2016). Additionally, the decreased biomass means that the availability of resources is restricted and activity will be more intense and shorter. The distribution of bacteria over the carcasses (Figure 3) also strongly shows that the substrate type and endogenous microbiota play an important role in the colonisation process. The heat map shown in figure 2 (averaged value of the bacterial abundance over all the carcasses for each day) shows a clear succession pattern. Initially, a population of *Pseudomonas* swarmed the samples (Days 1-3) and increased with time on the samples in mid to late decomposing stages (Days 7-10), while populations of *Bacillus licheniformis* and *Escherichia coli* increased throughout the decomposing stages. The transition between these stages in this particular study of progression was orderly, and comparable to observations from more extensive necrobiome studies in more temperate environments (Harrison et al., 2020; Cobaugh et al., 2015), but the rapid progression in this study points to a role for tropical climate conditions. The high humidity and warmth that prevail in southern Nigeria probably led to more rapid microbial turnover. Studies in cooler climates, however, have found more gradual microbial successions, because, temperature limits microbial growth, and activity

(Metcalf et al., 2016). *Pseudomonas*, *Bacillus* and *Staphylococcus*, have been consistently found in all types of carrion, indicating the presence of a core decomposer microbiota, that is able to dominate carrion, in a variety of substrates. They are important functional components of early and intermediate decay process because of their ability to survive under changing oxygen levels and metabolic versatility (Procopio et al., 2019). Concurrently, the compositional differences displayed in Figure 4 suggest specific microbial signatures found in the carcass of each of these groups; *Pseudomonas* was the dominant group in fish, with a greater proportion of *Staphylococcus* in birds, and a more even group in terrestrial carcasses. This selective colonisation is in line with an understanding that microbial communities are influenced by not only environment but also by chemistry of the carcass, intrinsic microbiota and tissue structure (Dangerfield et al., 2020; Wallace et al., 2021). The present study, however, adds data from an under-studied tropical environment and from a variety of vertebrate substrates simultaneously exposed to environmental conditions that address gaps in previous studies.

There are some caveats. Culture based methods only detect those that are viable and cultivable, which excludes obligate anaerobes and many fastidious organisms. Independent carcass replication and triplicate plating improved the data set, but future studies with molecular methods (16s rRNA sequencing and metagenomics) would give more detailed taxonomic information. Furthermore, although the trends in succession are similar on all the carcasses, the exploratory nature of the study does not allow for inferential statistical generalization. The results are thus patterns that are only ecologically informative and not formally predictive models.

5.0. Conclusion

In this study, it was shown that under tropical conditions in the field, bacterial succession occurs along predictable paths, depending on the stage of the process, and is strongly influenced by the identity of the carcass. The use of replicated carcasses, daily sampling and triplicate microbiology assays offers strong culture-based evidence for the dynamics of the necrobiome in a humid tropical environment. The results provide a regional baseline and the importance of substrate specific and environmental influences on decomposition processes. Future studies incorporating molecular approaches and multivariate analysis will be essential to further resolve microbial community complexity and enhance the application of necrobiome data in ecological and forensic context.

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Data Availability

The data generated and analysed during the course of this study are available from the corresponding author upon reasonable request.

Author Contributions

This author is responsible for the conception and design of the study, field data collection, data analysis, interpretation of results and preparation of the manuscript.

Ethical Approval

This study involved the use of animal carcasses for ecological decomposition research. All procedures were conducted in accordance with accepted ethical standards for ecological field studies and did not involve live animal experimentation.

Conflict of Interest

This author declares that there are no conflict of interests regarding the publication of this manuscript

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