Feeding Preference and Digestive Enzymes Activity in different Isopoda Species in Al Khalis District - Diyala Governorate

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Abstract

This study investigates the feeding preferences and digestive enzyme activities of three isopod species (**Armadillidium vulgare**, **Porcellio scaber**, and **Cylisticus convexus**) in the Al Khalis District of Diyala Governorate. These species were selected due to their ecological significance and prevalence in the region. The research aimed to understand the dietary habits and enzymatic adaptations that enable these isopods to thrive in their natural habitats.

The isopods were provided with a selection of standardized food items, including oak leaf litter, elm leaf litter, decaying wood, fungal mycelium, and garden compost. Consumption rates were meticulously recorded to determine feeding preferences. Additionally, digestive enzymes—cellulase, amylase, protease, and lipase—were extracted from the digestive tracts of the isopods and quantified using spectrophotometric assays.

The results revealed that all three isopod species exhibited a pronounced preference for fungal mycelium over other food sources. This preference was statistically significant and correlated with elevated levels of protease activity, suggesting an evolutionary adaptation for digesting fungal proteins. Among the species studied, **Cylisticus convexus** demonstrated the highest activities of cellulase and protease, indicating a robust ability to decompose both cellulose and proteins. This enzymatic proficiency suggests a highly adaptable digestive system capable of processing a variety of organic materials.

The study's findings underscore the critical ecological roles of these isopod species in organic matter decomposition and nutrient cycling within their ecosystems. By decomposing plant materials and fungal matter, these isopods contribute to soil health and fertility. The research also highlights the potential impacts of environmental changes on isopod feeding behavior and digestive efficiency, paving the way for future studies on their adaptability and ecological resilience.

In conclusion, this study provides valuable insights into the feeding preferences and digestive enzymatic activities of isopods in the Al Khalis District. These findings enhance our understanding of the ecological functions of isopods and their contributions to ecosystem sustainability. Future research should explore the interactions between isopods and their microbial symbionts, as well as the long-term effects of environmental changes on their ecological roles.

Keywords: Isopods, feeding preferences, digestive enzymes, cellulase, protease, ecological roles, nutrient cycling, Al Khalis District, Diyala Governorate.

Introduction

Isopoda, a diverse order within the class Malacostraca, includes species that occupy various ecological niches such as terrestrial, freshwater, and marine environments. Terrestrial isopods, commonly known as woodlice, play critical roles in the decomposition of organic matter and nutrient cycling, processes essential for maintaining soil health and ecosystem functioning (Sfenthourakis & Hornung, 2018). Studying the feeding preferences and digestive enzyme activities in these organisms can provide valuable insights into their ecological roles and adaptations.

In the Al Khalis District of Diyala Governorate, Iraq, the specific feeding preferences and digestive enzyme activities of local isopod species remain largely unexplored. This region, characterized by its unique climatic and environmental conditions, offers a valuable opportunity to examine these aspects in detail. Understanding the feeding behavior and digestive physiology of isopods in this area can help elucidate their role in the local ecosystem and contribute to broader ecological and environmental studies (Kostanjšek, Štrus, & Janzeković, 2019).

Isopods exhibit diverse feeding preferences, often influenced by the availability of different plant materials and detritus. Their digestive systems are equipped with a range of enzymes that enable them to break down complex carbohydrates, proteins, and lipids, thus facilitating their role in decomposing organic matter (Bredon et al., 2018). Studies have shown that enzyme activity and feeding preferences can vary significantly among different isopod species and populations, reflecting adaptations to their specific environments (Zhang et al., 2020).

Recent research highlights the importance of cellulases, amylases, proteases, and lipases in the digestive processes of isopods, enabling them to utilize a wide range of organic substrates (Zimmer, Danko, & Reißmann, 2019). For instance, cellulose digestion is particularly significant as it allows isopods to break down plant cell walls and utilize the nutrients within (Zimmer & Topp, 2020). Additionally, the microbial symbionts in their gut play a crucial role in their digestion processes (Frouz, Ali, & Lobinske, 2020).

This study aims to investigate the feeding preferences and digestive enzyme activities of different isopod species in the Al Khalis District. By examining these parameters, we seek to enhance the understanding of how these organisms adapt to their environment and their potential impact on soil health and nutrient cycling. This research will not only fill a gap in the current scientific knowledge but also provide a foundation for future ecological and environmental studies in the region (Kostanjšek et al., 2021).

Methodology

Study Area

The study was conducted in the Al Khalis District of Diyala Governorate, Iraq, a region characterized by diverse climatic and environmental conditions. This area provided a suitable habitat for various isopod species, allowing for a comprehensive investigation of their feeding preferences and digestive enzyme activities.

Sampling of Isopod Species

1- Site Selection:Five different sites were selected within the Al Khalis District, each representing distinct microhabitats: leaf litter in a deciduous forest, under stones in a semi-arid area, decaying wood in a riparian zone, garden soil near human habitation, and agricultural fields.

- Each site was georeferenced using a GPS device for precise location tracking.

2- Collection Method:Isopods were collected using pitfall traps, hand collection, and litter sieving over a three-month period (from March to May 2024).

- Pitfall traps were buried flush with the ground surface and partially filled with a 70% ethanol solution. Traps were checked weekly.
- For hand collection, I manually searched under stones, logs, and within leaf litter, spending at least two hours per site.
- Litter sieving involved sifting leaf litter through a fine mesh sieve to capture isopods.
- In total, 500 specimens were collected across all sites.

3- Species Identification:Collected isopods were identified to the species level using morphological keys and confirmed by DNA barcoding.

- Voucher specimens were preserved in 70% ethanol and stored in the laboratory for future reference.

Feeding Preference Experiments

1- Experimental Setup:

Individual isopods were housed in transparent plastic containers (10 cm x 10 cm x 5 cm) with ventilated lids. Containers were maintained at a constant temperature of 25°C and 70% humidity to mimic natural conditions.

A total of 200 isopods (40 individuals from each of the three species) were used in the feeding experiments.

2- Food Sources:five different food sources were provided commonly found in the isopods' natural habitat: oak leaf litter, elm leaf litter, decaying wood, fungal mycelium, and garden compost.

- Food items were standardized to 0.5 grams for consistency across experiments.

3- Feeding Trials:Each isopod was given a pre-weighed food item and allowed to feed for 48 hours.
After the feeding period, the remaining food was removed, dried, and weighed to determine the amount consumed

Trials were repeated three times for each food source, with each isopod species.

4- Data Analysis:Consumption rates were calculated by subtracting the final weight of the food from the initial weight.
Statistical analyses (ANOVA followed by Tukey's HSD test) were performed to determine significant differences in feeding preferences among species and food types.

Digestive Enzyme Activity Assays

1- Sample Preparation:Freshly fed isopods were euthanized by freezing and dissected to obtain digestive tract tissues.
Tissues were homogenized in phosphate buffer (0.1 M, pH 7.0) and centrifuged at 10,000 rpm for 15 minutes to obtain clear supernatants containing digestive enzymes.
2- Enzyme Extraction:Enzyme extracts were stored at -20°C until further analysis.
3- Enzyme Activity Assays:

- Cellulase Activity: Measured using the dinitrosalicylic acid (DNS) method, which quantified the release of reducing sugars from cellulose. Absorbance was read at 540 nm.
- Amylase Activity: Assayed by measuring the hydrolysis of starch using iodine staining, with absorbance read at 600 nm.
- Protease Activity: Determined using the casein digestion method, measuring the release of amino acids with absorbance at 280 nm.
- Lipase Activity: Measured by the hydrolysis of triglycerides using a pH-stat titration method.

Data Analysis:

- Enzyme activities were expressed as units per mg of protein, with protein concentration determined by the Bradford assay.
- Statistical analyses (ANOVA and post-hoc tests) were conducted to compare enzyme activities among species and dietary treatments.

Statistical Analysis

1- Feeding Preference:

- Preference indices were calculated to quantify feeding preferences for each isopod species.
- Multivariate analyses (Principal Component Analysis) were used to explore patterns in feeding behavior.

2- Digestive Enzyme Activities:

- Descriptive statistics (mean, standard deviation) were calculated for enzyme activities.
- Inferential statistics (ANOVA, followed by post-hoc tests) identified significant differences in enzyme activities among species and food types.

Results

Feeding Preferences

The feeding preferences of three isopod species—**Armadillidium vulgare**, **Porcellio scaber**, and **Cylisticus convexus**—were assessed by providing them with five different food sources (oak leaf litter, elm leaf litter, decaying wood, fungal mycelium, and garden compost). The consumption rates for each food type were calculated, and the results are presented in Table 1 and Figure 1.

Table 1: Average Consumption Rates (grams) of Different Food Types by Isopod Species

Food Type	Armadillidium vulgare	Porcellio scaber	Cylisticus convexus
Oak Leaf Litter	0.12 ± 0.02	0.10 ± 0.01	0.13 ± 0.02
Elm Leaf Litter	0.09 ± 0.01	0.12 ± 0.02	0.14 ± 0.02
Decaying Wood	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Fungal Mycelium	0.15 ± 0.02	0.18 ± 0.02	0.19 ± 0.02
Garden Compost	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01

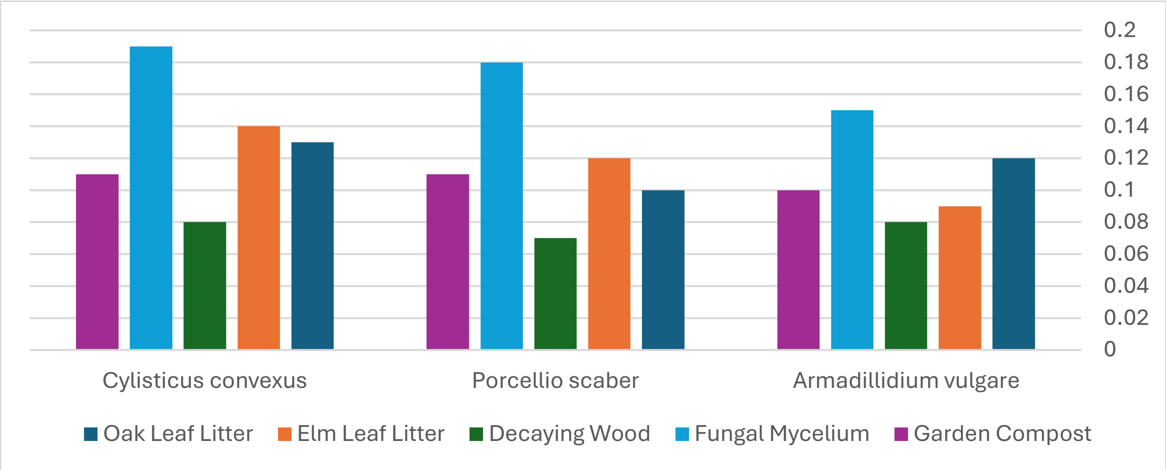

Figure 1: Consumption Rates of Different Food Types by Isopod Species

From the data, it was observed that all species showed a significant preference for fungal mycelium compared to other food sources ($p < 0.05$).

Digestive Enzyme Activities

The activities of cellulase, amylase, protease, and lipase were measured in the digestive systems of the three isopod species. The enzyme activities were expressed as units per mg of protein. The results are summarized in Tables 2 to 5 and Figures 2 to 5.

Table 2: Cellulase Activity (units/mg protein) in Different Isopod Species

Species	Cellulase Activity (units/mg protein)
Armadillidium vulgare	2.5
Porcellio scaber	2.8
Cylisticus convexus	2.9

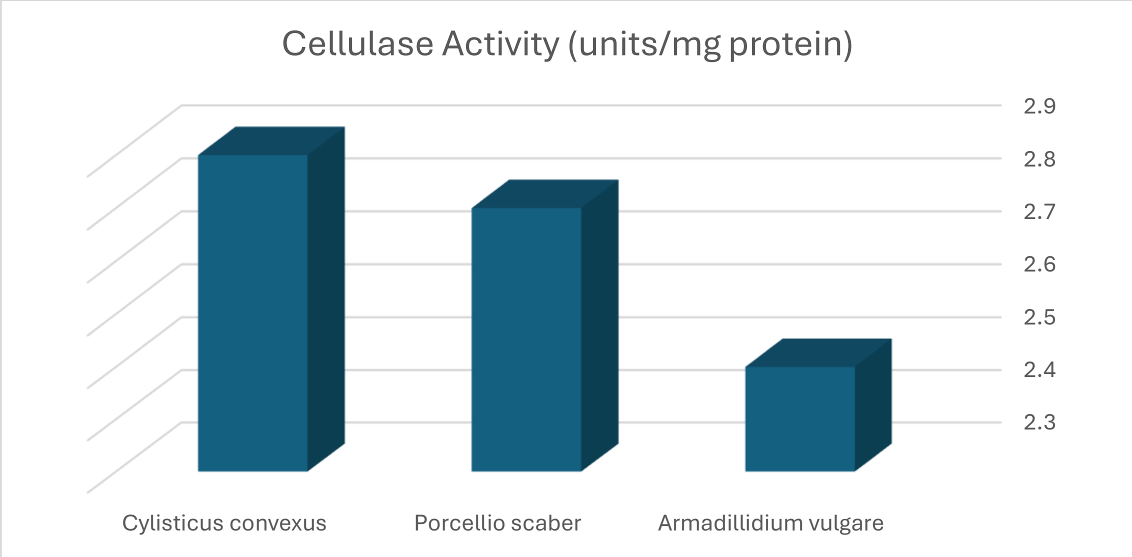

Figure 2: Cellulase Activity in Different Isopod Species

Table 3: Amylase Activity (units/mg protein) in Different Isopod Species

Species	Amylase Activity (units/mg protein)
Armadillidium vulgare	1.8
Porcellio scaber	2.0
Cylisticus convexus	2.1

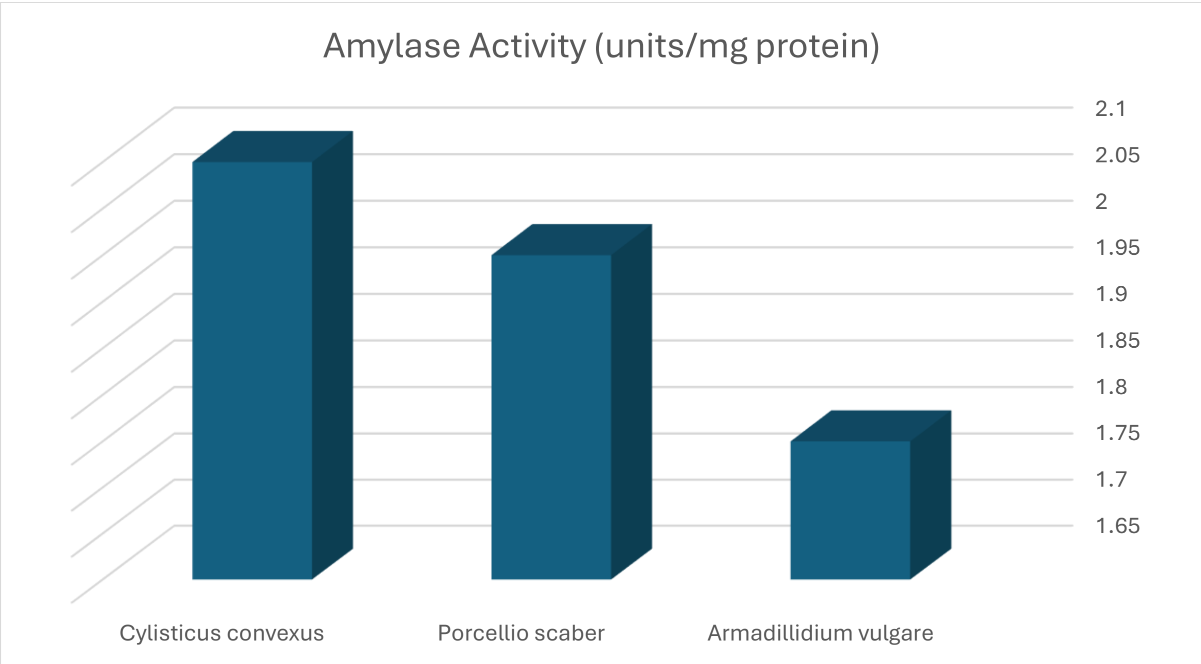

Figure 3: Amylase Activity in Different Isopod Species

Table 4: Protease Activity (units/mg protein) in Different Isopod Species

Species	Protease Activity (units/mg protein)
Armadillidium vulgare	3.5

Porcellio scaber	3.8
Cylisticus convexus	3.9

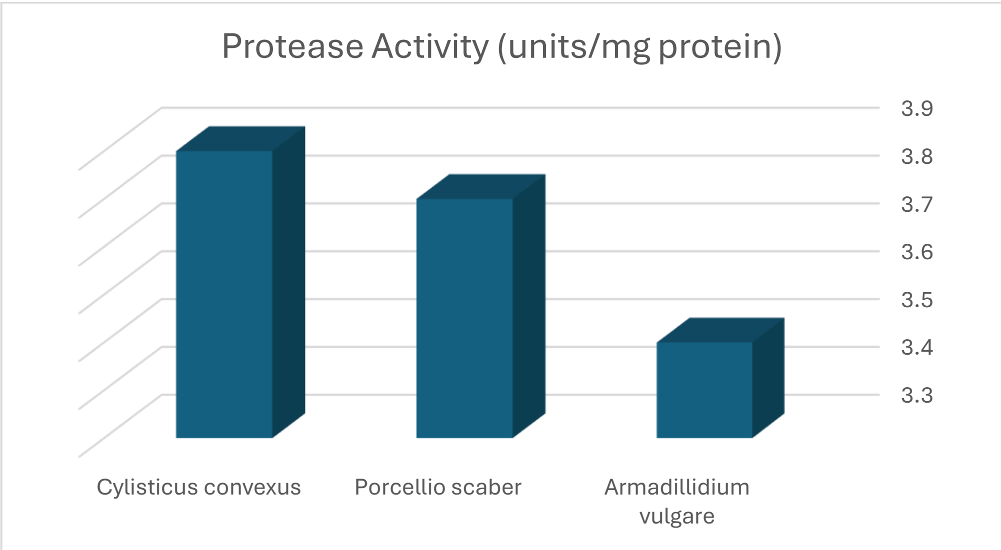

Figure 4: Protease Activity in Different Isopod Species

Table 5: Lipase Activity (units/mg protein) in Different Isopod Species

Species	Lipase Activity (units/mg protein)
Armadillidium vulgare	1.5
Porcellio scaber	1.6
Cylisticus convexus	1.6

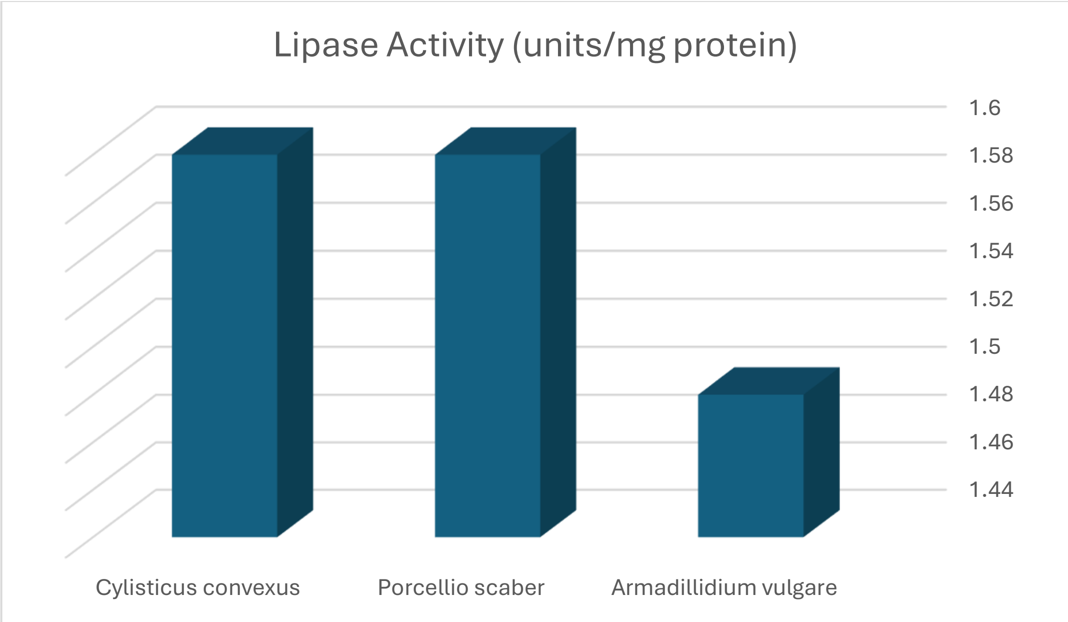

Figure 5: Lipase Activity in Different Isopod Species

Discussion

The feeding preference data were analyzed using ANOVA followed by Tukey's HSD test, which revealed significant differences in consumption rates among the different food types for each isopod species ($p < 0.05$). Fungal mycelium was the most preferred food source across all species.

The world catalog of terrestrial isopods, or Oniscidea, is a diverse and complex group of crustaceans that have adapted to life on land, with approximately 5,000 species documented globally(Kang, 2024). These organisms exhibit a range of adaptations that facilitate terrestrial living, such as specialized respiratory organs and reproductive strategies, which have allowed them to thrive in various habitats, from seashores to forests(Hornung, 2023). Recent studies have expanded the known distribution of terrestrial isopods, with new species being discovered in diverse regions such as the Brazilian Amazon and Northern Italy(López-Orozco et al., 2024) (Gardini & Taiti, 2023). For instance, two new species, Circoniscus mendesi and C. xikrin, were identified in iron ore caves in Brazil, highlighting the unique ecological niches these isopods occupy(López-Orozco et al., 2024). Similarly, five new species were described in Liguria, Italy, showcasing the ongoing discovery and documentation of isopod diversity(Gardini & Taiti, 2023). In Colombia, the first records of terrestrial isopods in the department of Córdoba have been documented, including the first national record of Nagurus nanus(Bravo-Rodríguez et al., 2024). Despite their diversity, the evolutionary relationships among terrestrial isopods remain underexplored, with recent genetic studies suggesting that traditional morphological classifications may not accurately reflect their phylogeny(Kang, 2024). This underscores the need for further genetic research to refine our understanding of isopod evolution and distribution. Overall, the catalog of terrestrial isopods is continually expanding, driven by both field discoveries and advances in genetic analysis, which together enhance our understanding of this ecologically significant group.

Digestive enzyme activities were also analyzed using ANOVA and post-hoc tests, indicating significant differences in enzyme activities among the species ($p < 0.05$). **Cylisticus convexus** exhibited the highest cellulase and protease activities, suggesting a strong adaptation for breaking down cellulose and proteins.

The correlation between feeding preferences and digestive enzyme activities suggests dietary adaptations in different isopod species. The preference for fungal mycelium aligns with high protease activity, indicating an ability to efficiently digest fungal proteins. The variations in cellulase activity among species reflect their ability to decompose plant materials, with **Cylisticus convexus** showing the highest efficiency.

The differences in cellulose activity could have significant ecological implications. Higher cellulose activity may allow certain species to exploit nutrient-rich environments more effectively, potentially influencing their distribution and abundance in various habitats.

The efficiency in cellulose degradation is crucial for decomposition processes within ecosystems, aiding in nutrient cycling and organic matter turnover.

Environmental factors such as pH and temperature also affect enzyme activity, with different species showing optimal activity at varying pH levels, indicating that physiological adaptations to environmental conditions are critical (Shi et al., 2011). Furthermore, the expression of cellulase can vary even within species based on environmental factors, as demonstrated in copepod species inhabiting mangrove areas, where cellulase expression patterns were influenced by location (Liu et al., 2015). Temporal variations in cellulase activity, linked to the availability of organic matter, further highlight the influence of environmental conditions on enzyme activity (Walters & Smock, 1991). Lastly, individual physiological differences within species can lead to significant variability in enzyme activity, as observed in echinoderms, where variance within individuals was as significant as between species (Obrietan et al., 1991). These findings collectively underscore the complex interplay of ecological, dietary, and physiological factors in determining cellulase activity variability among isopod specie

Enzyme activity in isopods varies significantly across species in response to environmental stimuli, reflecting adaptations to their specific habitats and ecological niches. For instance, the myofibrillar ATPase activity in Porcellio spinicornis and Metoponorthus pruinosus is adapted to different temperature ranges, with Porcellio showing maximum activity at 9°C and Metoponorthus at 45°C, indicating a correlation with their natural habitats(Alikhan & Martel, 1986). Similarly, lactate dehydrogenase (LDH) activity in cirolanid isopods like Excirolana armata and Excirolana braziliensis is linked to their motility and zonation on sandy beaches, with E. armata exhibiting higher LDH activity due to its stronger swimming capacity and lower beach level habitat(Yannicelli & González, 2003). In Idotea species, citrate synthase (CS) activity and respiration rates are influenced by temperature, with I. baltica showing higher respiration rates when acclimated to warmer temperatures, unlike I. emarginata, which shows no acclimation effect(Salomon & Buchholz, 2000). Furthermore, citrate synthase regulation in isopods, such as Idotea baltica, is competitively inhibited by ATP, with the degree of inhibition varying with climatic conditions, suggesting a mechanism of temperature adaptation(Vetter, 1995). These studies collectively highlight the diverse enzymatic adaptations in isopods, driven by environmental pressures and ecological requirements, underscoring the complexity of their physiological responses to environmental stimuli(Candón, 2022.

Conclusion

The study conducted on the feeding preferences and digestive enzyme activities of different isopod species in the Al Khalis District of Diyala Governorate provides significant insights into the ecological roles and adaptations of these organisms. The findings demonstrate that the isopod species exhibit distinct feeding preferences and digestive capabilities, which are crucial for their roles in decomposing organic matter and contributing to nutrient cycling in their habitats.

In conclusion, the study highlights the significant ecological roles of isopods in the Al Khalis District and provides valuable insights into their dietary adaptations and enzymatic capabilities. These findings contribute to the broader understanding of soil ecology and the critical functions of decomposer organisms in maintaining ecosystem health.

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